

Mesenchymal Diseases in Childhood

Report of the Twenty second Ross

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Introduction

In the mesenchymal diseases often referred to as collagen diseases the diffuseness of our knowledge of etiology and pathogenesis has long hindered our efforts to arrive at satisfactory classification and effective treatment. The search for clarification has led down many blind alleys, and doubtless many more will be traversed.

These proceedings discuss current concepts through which we approach these conditions. Recently developed techniques have enabled us to interrelate earlier findings with data derived from the application of these techniques, raising new problems as well as laying to rest some of the old. Classification has been made easier and with increased understanding of the natural course of mesenchymal diseases treatment has been made more effective.

I am sure that the pooling of information among the many investigators that have been brought together here will increase the effectiveness of all in research and in clinical application.

Vincent C. Kelley
Chairman

General Considerations in Mesenchymal Disease

Role of Infection in the Etiology of Mesenchymal Diseases

DR CHANDLER A. SYETOV The etiology of rheumatic fever has variously been explained by virus infection, nutritional deficiency and other theories that now seem bizarre. The association between rheumatic fever and infection with the hemolytic streptococcus was so evident by the 1920's, however, that continued research in that direction has eventually led to our present knowledge of etiology. The pathogenesis of rheumatic fever may well remain obscure until the disease is successfully reproduced in animal, but it seems certain that present studies are aided enormously by our knowledge that the group A beta hemolytic streptococcus is somehow the etiologic agent. From this knowledge have also come the therapeutic and prophylactic measures by which we can now effectively prevent rheumatic fever and its recurrences.

With the demonstration that only certain serologic types of streptococci, especially type 12, cause acute glomerulonephritis, the etiology of this disease also seems to be satisfactorily established. It is unlikely that we shall solve the problem of the pathogenesis of other mesenchymal diseases or find effective measures for controlling them until their etiology is known.

Experimental polyarthritis

Clinicians and pathologists were once ready to accept as a working approach an infectious etiology in rheumatoid arthritis. There was apparently no difficulty in reproducing analogous disease in experimental animal. A high proportion of rabbits developed chronic polyarthritis after intravenous inoculation with streptococci. Chronic polyarthritis clinically and pathologically much like rheumatoid arthritis, was produced by infecting mice with pleuropneumonia like organisms. The presence of elevated titers of streptococcal agglutinins in the serum and/or fluid of the joints of patients with rheumatoid arthritis was repeatedly demonstrated.

Subsequently, experimental polyarthritis was shown to differ from the clinical disease in important respects. The streptococci or

pleuropneumonia like organisms could be recovered from the infected joints in the experimental disease while cultures from joints of patients with rheumatoid arthritis were usually sterile. The presence of antistreptococcal antibody seemed less significant with the discovery of the sheep cell agglutinating factor and the other peculiar properties of the serum of these patients. The demonstration that the titer of antistreptolysin O was not elevated seemed to exclude the hemolytic streptococcus as the etiologic agent.

Statistically valid studies did not disclose significant incidence of septic foci in patients with rheumatoid arthritis. Finally the failure of antibiotics to affect the course of the disease caused the majority of workers to abandon the general notion of an infectious etiology for rheumatoid arthritis although some still clung to the possibility of a viral etiology. In recent times rheumatoid arthritis has been thought to be a disease of adaptation of hypersensitivity of collagen of the liver, thyroid, et cetera.

Studies of the components of connective tissue and new advances in the field of endocrinology have largely replaced the earlier bacteriologic and experimental pathologic approaches in studies of rheumatoid arthritis. However despite the advancement of several ingenious and brilliant hypotheses little real insight into the etiology seems to have emerged from either of these new lines of approach. Once the etiology of rheumatoid arthritis is known the vast amount of biochemical and neuroendocrinologic information now being obtained will undoubtedly make the subsequent steps in elucidation of pathogenesis quicker and more certain.

L forms of bacteria

For the past 10 or 15 years a new development in bacteriology has concerned the peculiar dissociants of bacteria known as L forms. Various bacteria including the hemolytic and nonhemolytic streptococci when grown under certain unfavorable conditions shed their capsules, cell walls, and most of their cytoplasmic constituents and exist as free living, slowly reproducing organisms apparently consisting largely of nucleoprotein. The techniques of culturing the L forms are sufficiently rigorous to discourage most investigators. However the basic experiments have been repeatedly confirmed and the work may well come to be regarded among the outstanding advances in microbiology of our time.

The L forms are in some cases morphologically indistinguishable from the pleuropneumonia like organisms found in the human genital tract and in animals with certain diseases. While there has been little study of the pathogenicity of L forms the extreme specificity of pleuropneumonia like organisms for certain tissues of some species is well known.

It has been suggested that the minute pleuropneumonia like L forms would be invisible in tissues. Furthermore it is likely that conditions favorable for transformation to L forms probably exist during bacterial infection and it is possible that during convalescence from infection the presence of antibody and other participants in the host's defenses may lead bacteria to abandon their antigenic and vulnerable cell wall and become L forms.

If L forms were present in the joints it is unlikely that they would have been demonstrated by cultural techniques previously used. L forms of streptococci are highly resistant to penicillin and because they do not produce streptolysin O we would not expect elevated titers of antistreptolysin O. We would expect on the other hand to find antistreptococcal α and β globulins in the serum of patients with rheumatoid arthritis, since these are primarily active against the nucleoproteins that make up much or most of the substance of the L forms.

The high incidence of rheumatoid arthritis in patients with agammaglobulinemia suggests that the repeated infections experienced by these patients may have played an etiologic role and it would seem that any new appraisal of the role of infection in rheumatoid arthritis should include a study of streptococcal L forms.

Pathogenesis

It seems clear on the basis of bacteriologic, serologic and epidemiologic evidence that the group A streptococcus plays a causative role in rheumatic fever but the mechanisms by which it produces the rheumatic complication are as much a mystery as ever. The possibility of a direct streptococcal infection of cardiac and other deep tissues remains a real one. Since rheumatic fever can apparently be prevented by adequate therapy with penicillin even if this therapy is delayed for 10 days after the onset of streptococcal pharyngitis it seems that living streptococci must be present in the body at the time rheumatic fever begins and not merely two or three weeks previously.

A concept that has received wide acceptance is that bacterial allergy or delayed hypersensitivity to streptococci is basic in the pathogenesis of this disease. When skin testing with streptococcal nucleoproteins is carried out in patients with acute streptococcal pharyngitis it is found that the great majority have positive delayed type skin reactions three or four weeks after the onset of the disease. There seems to be no correlation between the size of the reaction in the skin and the amount of circulating gamma globulin or antistreptolysin O and there is still no direct evidence implicating hypersensitivity in this disease.

Another possibility in the pathogenesis of rheumatic fever is that endotoxins are somehow involved. It has been demonstrated that bacterial endotoxins are capable of producing damage in tissues resembling that seen in certain of the mesenchymal diseases but until recently it was believed that only gram negative bacterial species elaborate endotoxins. We have found however that group A streptococci contain an analogous material that produces in normal animals all the effects hitherto demonstrated for gram negative bacterial endotoxins including the production of intravascular fibrinoid. It is not yet known whether the biologic activity of this material is involved in any of the nonsuppurative complications of human streptococcal infections but this is a challenging possibility that would seem to deserve exploration.

As with rheumatic fever the pathogenesis of acute glomerulonephritis is still obscure. The search for some nephrotoxin produced by the type 12 streptococcus seems to be a chief line of approach. Another deals with the possibility that lysis by bacteriophage may occur with sudden dumping of large amounts of antigenic material into the body. Hypersensitivity of either the circulating antibody type or of the delayed type may be involved but there is no direct evidence for this.

In summary then the role of infection in mesenchymal disease seems well established in at least two entities acute rheumatic fever and acute glomerulonephritis and it seems possible that further study might establish its importance in others.

Role of Allergy in Mesenchymal Diseases

General consideration of allergic responses

DR WILLIAM E. EHRLICH Allergic responses or allergies as they occur in human beings and in animals may be considered to fall into two major groups—the immediate types and the delayed type. Among the immediate or early responses are the wheal and erythema type and the Arthus reaction or anaphylactic type. Anaphylactic sensitivity is transmitted by antibodies in the serum which are precipitated by antigen. These antibodies are gamma globulins synthesized by plasma cells. Patients who cannot produce gamma globulin because they have no plasma cells are not able to develop this hypersensitivity.

Responses of the wheal and erythema type can be transferred to other individuals by antibodies in the serum known as sensitizing antibodies or atopic reagins. These antibodies differ from others in that they cannot be demonstrated by precipitation with antigen although they can be demonstrated by the Prausnitz-Kustner reaction. They do not pass through the placenta as more common antibodies do, suggesting a rather large molecular weight. It now appears that these antibodies may also be formed by patients with agammaglobulinemia.

Delayed hypersensitivity is characterized by the absence of antibodies in the serum. Hence it cannot be transmitted or transferred by serum but it can be transferred by cell. Recent reports indicate that injections with BCG or other tuberculin material produce this tuberculin type of sensitivity in patients with agammaglobulinemia.

Clinically the wheal and erythema response is manifested in typical cases by the appearance of hay fever, asthma, hives, and urticaria. The anaphylactic type of allergy is characterized most often by serum sickness, while the delayed response is usually obscured by the manifestations of underlying infectious disease. However, in the sensitivity produced by poisoning, the direct response of this delayed type of allergy may be seen in the tissues.

It should be emphasized that these responses are not mutually exclusive. Skin sensitizing antibodies and precipitins often occur concomitantly and clinical and morphologic manifestations often suggest the presence of more than one type of allergy.

Morphologic reactions in tissues in mesenchymal diseases

Morphologically the reactions in the tissues in the various mesenchymal diseases are very much the same. For instance, in mild or acute cases there may be mucoid degeneration (figure 1 upper left) that is swelling of the connective tissue due to accumulation of ground substance. Serous inflammation (figure 1 upper right) too may occur in acute stages. This differs from mucoid degeneration by accumulation in the tissue of an edema fluid containing rather high concentrations of protein including enzymes and enzymatic activators. The latter may cause lysis of elements of the connective tissue called demolysis.

If the lesion is of greater severity, larger molecules such as fibrinogen pass through the capillary wall and are precipitated in the connective tissue in the form of fibrinoid, a process known as fibrinoid degeneration (figure 1 lower left). Fibrinoid apparently differs from fibrin in that the precipitate includes other substances

along with fibrinogen which can be demonstrated in tissues with ordinary histologic techniques. There may be other proteins involved and carbohydrates, fats and even nucleic acids may be precipitated. Fibrinoid is not a uniform substance and it is clear that there are various fibrinoids all of which probably contain fibrinogen.

In the most severe cases the deposition of fibrinoid may be associated with necrosis referred to as fibrinoid necrosis. If the collagen disease is chronic we usually do not see retrograde or



Figure 1 Morphologic reactions in tissues characteristic of mesenchymal diseases are (upper left) mucoid degeneration (upper right) serous inflammation (lower left) fibrinoid degeneration and (lower right) granuloma to it. Reprinted from The Journal of Experimental Medicine 89: 3 (January) 1919.

regressive changes but rather productive or proliferative changes known as granulomas (figure 1 lower right). Some of the granulomas that develop in diseases of connective tissues are highly specific. The Aschoff bodies for example are so characteristic that they are diagnostic of the disease. However in many other cases the granulomas are non specific.

The wheal and erythema response is characterized by serous inflammation. The vessels become extremely permeable and components of plasma leak into the tissue. On the other hand the Arthus or anaphylactic response is characterized by severe fibrinoid degeneration and necrosis especially of the arteries while the delayed response is characterized by the formation of granulomas.

The outcome of the various reactions that we see in the collagen diseases is quite uniform. Mucoid degeneration may resolve spontaneously without leaving any scar or other evidence of existing disease. On the other hand serous inflammation, fibrinoid degeneration and necrosis and granulomatous result in sclerosis or scar formation with permanent damage.

The involvement of the connective tissue in these various diseases is frequently associated with similar reactions of the vessels, more frequently the arteries, suggesting that the noxious agent was present in the circulating blood and entered from there into the arterial wall. Thrombosis is commonly observed in the vessels and the composition of the thrombi appears to be as variable as the composition of the fibrinoid.

Ten to twenty days after the first injection of large doses of horse serum, proliferative changes develop in the vascular connective tissue with swelling of the musculature and proliferation but not degeneration of cells. If the injection is repeated, a sudden clash of large quantities of antigen and antibody in or on the vascular connective tissue results in fibrinoid necrosis. In many cases in which proliferative lesions develop from the first injection, degenerative lesions appear after the second injection.

Allergic factors in pathogenesis of specific mesenchymal diseases

The changes in the tissues characteristic of collagen diseases are generally identical to those seen in serum sickness. Although serum sickness is known to be an allergic response, the collagen diseases are not necessarily of allergic nature. Fibrinoid degeneration and perhaps other changes seen in these diseases can be elicited by other mechanisms. However, the lesions in the so-called collagen diseases are consistent with the possibility of allergic origin.

A disease resembling rheumatic fever has been produced experimentally by the induction of successive infection with different types of group A hemolytic streptococci. The fact that the serum of most patients with rheumatoid arthritis strongly agglutinates group A hemolytic streptococci and sensitized sheep cells has been offered as evidence that rheumatoid arthritis is a product of hypersensitivity. However, some investigators have reported that the agglutinins or reactants in these patients differ from antibodies in various ways. They cannot be suppressed by cortisone as are other antibodies and furthermore it has been shown that nonsensitized colloidal particles like case are agglutinated by these sera. I wonder whether these are not non-specific instabilities that occur in the disease in the colloid chemical status of the serum and may be also of

the connective tissues. Surely there is no direct evidence to prove that these instabilities are caused by true antigen antibody reactions or any other allergic mechanisms we know of.

There is no evidence as yet that systemic lupus erythematosus, dermatomyositis or scleroderma are of allergic origin. On the contrary there has been a recent report of generalized scleroderma in a child with agammaglobulinemia.

Polyarteritis nodosa and serum sickness resemble each other so closely that they may be considered different expressions of the same process. An antigen antibody reaction of the anaphylactic type seems to be involved. However patients with the former disease frequently develop severe asthma, a manifestation of the wheal and erythema type of allergy.

We can conclude then that in some of the diseases of connective tissue an allergic nature has been definitely established. In others certain observations suggest that allergy may be a factor while in a third group at least the anaphylactic type of allergy can be definitely excluded.

Discussion

Development of mesenchymal diseases in patients with agammaglobulinemia

DR ROBERT A. GOOD: Ehrlich mentioned that we might learn something concerning the possible allergic etiology of mesenchymal diseases from interpretation of the experiments of nature presented by patients with agammaglobulinemia. These patients have a gross deficit in capacity to form circulating gamma globulin and have almost no circulating antibodies. We have observed two patients with agammaglobulinemia who have developed classic atopic dermatitis, a sensitivity reaction dependent on the nonprecipitable wheal and erythema type antibody. Recently I learned of a patient with agammaglobulinemia who had hay fever. Circulating nonprecipitable heat labile antibody was identified in significant concentration and this sensitivity could be transferred by injection of serum to a non sensitive recipient.

We have observed four patients with agammaglobulinemia who have developed typical rheumatoid arthritis. The incidence of rheumatoid arthritis in our series of patients with agammaglobulinemia is therefore considerably greater than the incidence in the population at large. Hansen studied a patient with congenital agammaglobulinemia, failure of the immune response who developed classical dermatomyositis.

Patients with α -amaglobulinemia can develop tuberculin reaction and can react to administration of typhoid paratyphoid antigen as do normal patients. Bacterial allergy against streptococcal products can be transferred to patients with α -amaglobulinemia and they are able to sustain this form of allergy. We have been able to induce in patients with agammaglobulinemia delayed allergic responses to certain chemicals and to transfer this delayed allergy to subject who have not had contact with the chemical.

It seems we should be less concerned with classic circulating antibodies as important pathogenetic agents in this group of disease and more concerned with the delayed allergy or possibly the allergy dependent on the heat labile nonprecipitating reagin type of antibody.

DR STETSON: Because patients with α -amaglobulinemia are extensively and energetically investigated I wonder whether some of the things done to them perhaps contributed in some way to the rather high incidence of mesenchymal and mesenchymoid diseases.

DR H. A. GOOD: Fortunately for us with our intensive investigation three of the four patients with agammaglobulinemia who have had rheumatoid arthritis had the disease at least two years before any studies were carried out.

Psychosomatic Aspects of Mesenchymal Disease

DR C. KNIGHT ALDRICH: In the development of any psychosomatic illness emotions participate as one of a group of causative elements which vary in significance as well as in response to treatment from individual to individual.

Emotional factors in the etiology of rheumatoid arthritis

The interrelation of emotional factor with other contributing causes in the etiology of rheumatoid arthritis in children is complex. Other participating factors are assumed to be of hormonal, inherited, traumatic or infectious nature. Personality characteristics, adaptation pattern and historic event similar to those found in the lives of patients with rheumatoid arthritis have been observed in patients with other psychosomatic disorders, certainly suggesting that emotional factor are not the only important determinants of

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the site of symptoms. Investigations of the significance of emotional factors are handicapped however by the chronicity and severity of the illness as well as by the lengthy and time consuming nature of psychiatric treatment. Thus it is difficult to differentiate the effects of treatment from the effects of events in the natural course of the patient's life and it is difficult for any investigator to treat a statistically significant number of patients.

In spite of these difficulties there is some evidence that emotional factors make both direct and indirect contributions to the development of rheumatoid arthritis, and some indication that similar factors affect other diseases of the mesenchymal group. The evidence accumulated by studying developmental histories and the personality structure of adults with rheumatoid arthritis points to a characteristic constellation of personality factors. These studies demonstrate persistent and powerful but unfulfilled dependent needs arising out of early disturbances in the mother-child relationship resulting in hostility towards the mother which is ordinarily channeled into a masochistic need to serve others. In other words the arthritic patient in the earliest stage of development has lost out on some of the gratifications he should have received from the dependent relationship with his mother. He therefore remains chronically insecure having stored up the child's equivalent of intense anger towards the mother who failed him. His fear of losing the small security he has prevents his expressing hostility directly. He learns that to serve others wins their approval and keeps his hostile feelings under control.

Sometimes however situations arise that either interfere with masochistic methods of dealing with hostile feelings or else mobilize guilt secondary to the hostility. These situations are a socialized in predisposed individuals with the onset or exacerbations of rheumatoid arthritis.

Electromyographic studies have provided suggestive evidence that the patient with rheumatoid arthritis is likely to reflect his psychologic tensions through somatic muscular tension. This evidence preliminary though it is supports the theory that persistent increased muscle tonus resulting from inhibited aggressiveness and the defenses against it in some way precipitates the attack of arthritis. Glaser has noted that when the emotional tension of patients with rheumatoid arthritis finds redirection in manifestations of psychosis the arthritic symptoms remain minimal. The alternation of psychosis and psychosomatic symptoms indicate the interrelationship of emotions and this type of symptom.

Surveying the results of their psychotherapy on the arthritic process in children Blom and Nicholls report their impression that with treatment remission comes sooner and lasts longer but state that

improvement can be better demonstrated in terms of general emotional adjustment than in alteration of arthritic symptoms

Emotional effects of mesenchymal diseases in children

The emotional effects of mesenchymal diseases in children can be discussed under three general headings, emotional effects of any chronic disease, implications of changes in the child's body image, emotional by products of treatment particularly of treatment with steroid hormones

Any disease that produces chronic invalidism subjects the patient to an unusual degree of dependency on his environment. The helplessness and restrictions imposed by illness may produce a variety of emotional reactions. Some children become particularly anxious, others are depressed, apathetic or withdrawn. Still others deny their incapacity, thus avoiding the necessity to face their dependency. The choice of reaction by a given child depends on the sum total of the relationships experienced and the attitudes developed in the earliest period of his life when dependency was inevitable.

When necessary, mature adults can accept the dependency of illness with equanimity, remaining eager and willing to give it up when their physical condition improves. This process is more difficult in children and immature adults. Early experiences have conditioned some to equate helplessness and dependency with abandonment or rejection, others have been conditioned to feel ashamed of any dependent yearnings. These attitudes contribute to the development of anxiety and depression during illness of any kind. Other children appear to be trying to capture a security they did not receive when they were younger, accepting illness reasonably well but resisting convalescence and seeking to maintain their dependent position.

The distortion of body parts brought on by illness is frightening enough to an adult but much more so to the child. The child who sees a joint becoming swollen, painful and stiff does not easily comprehend that the illness is likely to be limited to the joints but may have terrifying fantasies that the disability will in some way extend to other more vital parts.

The patient's concept of body image is important in the reaction to treatment, particularly by corticoid. It is difficult to establish in what proportion the biochemical effects of the corticoids and the anxieties associated with the modifications in body image contribute to the psychoses occasionally seen during treatment with these substances. The confusion in identity and the distortion of body image produced when an adolescent girl finds her face changing in shape

accompanied by the development of extensive acneform lesions and hirsutism may be more significant in producing a psychosis than the alterations in body chemistry. Assuming that both play a part it is particularly important in the interest of mental hygiene to provide constant reassurance, explanation and interpretation of the nature of the disease and the side effects of treatment to any child with mesenchymal disease.

Biochemical and Hematologic Alterations in Mesenchymal Disease

Some Chemical and Biologic Properties of Chondroitin-sulfuric Acid B

DR ALBERT DORFMAN In recent years many diseases have been thought to be associated with changes in ground substance. Because of the limited histochemical methods available interpretation of such changes has been unsatisfactory.

There is now sufficient evidence to indicate that ground substance is not a uniform chemical substance but rather a complex physicochemical or colloidal solution. This amorphous material separates fibers and cells and is thus the phase interposed between all parenchymal cells and circulation. The ground substance contains materials in transit between cells and circulation and substances peculiar to connective tissue. The composition of ground substance varies in different parts of the body and in different tissues. In articular synovial fluid and vitreous humor for example the composition appears to be considerably simpler than in skin.

Proteins of ground substance

The proteins of ground substance include acid soluble collagen, neutral soluble collagen, protein components of mucopolysaccharide complexes and possibly precursors of elastin.

The existence in vivo of acid soluble collagen in solution remains questionable. It is apparent from recent work that a soluble form of collagen called the neutral soluble fraction is present in large quantities in ground substance and has a rapid metabolic turnover.

Sulfated mucopolysaccharides exist in certain tissues as protein complexes of high molecular weight. The chemical linkage between protein and mucopolysaccharide is unknown. It seems likely that precursors of elastin should also be present in the ground substance.

obtained from the unknown compound behaved on paper chromatography just as that obtained from a known sample of *L* iduronic acid

It is of interest that iduronic acid has never been demonstrated to occur naturally. *D* glucuronic acid and *L* iduronic acid differ only in the stereoisomerism of the fifth carbon atom

Some biologic properties of chondroitin sulfuric acid B

Another approach to the study of this compound has concerned its biologic activity. Marbet and Winterstein have reported that the antithrombic activity of chondroitin sulfuric acid B is considerably less than that of heparin. However the former compound occurs in connective tissue in rather large amounts. 25 mg of the pure compound can be isolated from the skin of one rabbit but only minute amounts of heparin can be isolated from tissues. Even if the anticoagulant activity of chondroitin sulfuric acid B is weak compared to that of heparin the total activity might be great. Chondroitin sulfuric acid A demonstrates no antithrombic activity.

For study of relative antithrombic activity the basic design of the experiments carried out with Grossman consisted of incubating a mixture of purified thrombin with the particular compound in question and in most cases with plasma. After a period of incubation of 15 minutes an aliquot was mixed with fibrinogen and the clotting time determined. Increase in clotting time beyond that of the control indicates antithrombic activity.

It has long been known that heparin alone is not an antithrombic substance but requires for activity some other component present in plasma. There has been considerable disagreement on whether this component should be called a heparin cofactor or whether heparin itself should be considered the cofactor and the component in plasma the antithrombic substance.

With impure fibrinogen heparin appears to be active because of contamination with antithrombic substance. For this reason we utilized purified fibrinogen. Under the conditions chondroitin sulfuric acid B alone does not demonstrate antithrombic activity but when mixed with plasma the antithrombic activity of plasma is augmented. In the presence of plasma and at low concentrations of thrombin (10 to 20 units/ml) chondroitin sulfuric acid B has considerably more antithrombic activity than heparin. However at high concentrations of thrombin (200 units/ml) chondroitin sulfuric acid B does not exhibit antithrombic activity.

Figure 3 represents the results of an experiment indicating the relative antithrombic properties of plasma of plasma plus heparin and of plasma plus chondroitin sulfuric acid B. All clotting assays were performed at theoretically constant thrombin concentrations.

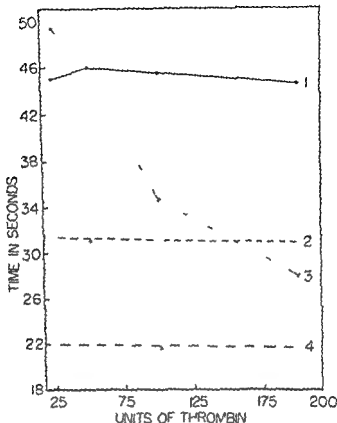


Figure 3 The relative activity of plasma plasma plus heparin and plasma plus chondroitin sulfate after incubation with various concentrations of thrombin. The aliquots of the incubation mixture were diluted before reaction with fibrinogen so that in each case the same amount of thrombin was used in determining the clotting time. Curve 4 represents the clotting time of the control mixture thrombin plus fibrinogen. Curve 3 indicates the effect of incubating the thrombin with plasma and Curve 1 indicates the effect of incubation of thrombin plasma and heparin. In these three curves it may be seen that the concentration of thrombin in the incubation mixture does not appear to influence the result. However when the mixture plasma and chondroitin sulfate are incubated together the clotting activity (Curve 3) is greatly increased when the concentration of thrombin in the incubation mixture is lowered, a result less than that of plasma alone when the concentration of thrombin in the incubation mixture is high.

but the thrombin concentration was varied in the thrombin plasma polysaccharide mixture. Plasma exhibits definite antithrombic activity that is augmented by the addition of heparin at all concentrations of thrombin tested. The addition of chondroitin sulfuric acid B to thrombin and plasma results in marked augmentation of antithrombic activity at low concentrations of thrombin. At high concentration of thrombin chondroitin sulfuric acid B is not only less active but partially neutralizes the antithrombic activity of plasma.

These data would seem to indicate that certain polysaccharides in ground substance may have highly specific activities that are quite different from those of other compounds closely related chemically. We do not know the exact chemical structure of chondroitin sulfuric acid B or anything about its metabolism. What role it may play in physiologic or pathologic conditions is not clear. It is clear however that there is a substance normally present that could profoundly affect the deposition of fibrinogen in the ground substance of connective tissue and it seems possible that the amount of such material present, its physicochemical state or its dissociation from proteins may well play some important role in both physiologic and pathologic processes.

Significance of Micelloghagosis in the Physiology of Ground Substance

DR. ROBERT D. HIGGINBOTHAM. I should like to review some studies concerning an interrelationship of the mucopolysaccharide of ground substance with cells of connective tissue in the maintenance of the physiologic state. They deal in large measure with the intracytoplasmic storage or sequestration of noxious foreign material, a collaborative function of ground substance and connective tissue cells that we have called micelloghagosis. Concerned in this process are the mast cells, the fibroblasts and of course ground substance—the amorphous continuum between the fibers, cells and vessels of the connective tissue.

The importance of micelloghagosis is implied by the interrelated roles of the connective tissue elements in the transport of metabolites and the maintenance of an optimal environment for cellular function. Changes in both chemical and physical states of the ground substance as well as of the cellular constituents of connective tissue are indicative of altered function and are characteristic of the inflammatory response.

Combination of mucopolysaccharides with noxious foreign materials

Ground substance may function in a manner analogous to that of ion exchange resins. The calcium binding properties of cartilage are well known and a selective affinity for the potassium ion has been demonstrated for the polymerized ground substance of the symphyseal pubis of the guinea pig. This postulated role of ground substance as an exchanger of ionizable substances would be governed in part by the number of acidic groups present and the degree of polymerization of the mucopolysaccharides as well as by the complex nature of the other constituents of ground substance.

We have tried to determine whether the binding of various substances by mucopolysaccharides may function in the presence of noxious agents as a mechanism for the maintenance of the physiological state in the connective tissue. A schematic illustration of this function is shown in table 2. The acidic mucopolysaccharide (MPs) binding with an appropriate substance X which may be an amine or protein of the connective tissue forms a complex with it. This complex in the presence of another substance Y may be dissociated forming a new complex (MPs - Y) and releasing X from the original complex. The substance Y would therefore be known as a releaser.

Preliminary studies have shown that a complex of heparin with toluidine blue may be dissociated by basic substances that have a strong affinity for this mucopolysaccharide. The second complex at optimal proportions forms a precipitate and the dissociated dye is freely dialyzable. The release of dye shows indirectly that the heparin has formed a new complex with the basic substance or toxic agent and thereby reduced the concentration of active toxic substance.

The relative strengths of these releasers were estimated from the amount required to release 25 to 50% of the toluidine blue from its metachromatic complex with heparin. A number of the strong and moderate releasers such as polyvinylpyrrolidone, neomycin and the chemotherapeutic diamidine compounds are therapeutic drugs whose use has been restricted because of the toxicity associated with them. Of the other substances, both 48/80 and basic lead acetate are reportedly very efficient releasers of histamine from the heparin-histamine complex in tissue.

After subcutaneous injection of heparin in the mouse metachromatic granules appeared in the local fibroblasts suggesting that the cells were able to ingest and concentrate this substance as intracellular granules. Injection of chondroitin sulfate P produced a similar although weaker effect than that of heparin.

Additional studies showed that chemically sulfated hyaluronic acid as well as chondroitin sulfate A appeared in the cells two hours

after injection. The cells that ingested these chemically altered mucopolysaccharides were unable to dispose of them and granules were seen in their cytoplasm for at least one month after injection in contrast to the 24 to 48 hours required for the appearance of naturally occurring mucopolysaccharides.

The sequestration of such altered or foreign material and the intracytoplasmic storage which we have termed micellophagosis suggests that the cellular function is also one of detoxification, the ingested material being no longer free to act extracellularly. The initial

Table 2

Acidic mucopolysaccharide exchange of basic substances

MPs X	+	Y	→	MPs Y	+	X
Heparin (50 µg)		Toluidine Blue (120 µg)	+	Releaser		(50 ml)
Heparin Releaser			+	Toluidine Blue		

Releasers			
STRONG	MODERATE	WEAK	INEFFECTIVE
48/80	Stilbamidine	Cadaverine	d tubocurarine
Polymyxin	I entamidine®	Putrescine	I yr benzamine®
Protamine	Propamidine®	Streptomycin	Tryptamine
Neomycin	Spermidine		Morphine
Clupeine	Ba sic I bAc		Glucosamine
ACTH			Ba tra in etc

Compared on basis of amounts necessary for dye release (DR)
 STRONG = 50% DR/25-100 µg MODERATE = 50% DR/50-1000 µg
 WEAK = 95% DR/1000 µg and INEFFECTIVE = 95% DR/1000 µg

inflammatory response subsides after a time despite the continued presence of the noxious substance in the tissues. The presence of dark metachromatic granules in the cytoplasm of the fibroblasts provides an appearance quite similar to that of the mast cell. We have described such cells as "quasi mast cells" to denote this superficial resemblance.

Further investigations included subcutaneous injection into mice of mixtures of heparin with various basic substances: heparin 48/80 complex, heparin stilbamidine complex, heparin polymyxin complex and heparin neomycin complex. It was observed that these substances which reacted with heparin in vitro to form a cloudy solution altered either the appearance of the granules or the rate at which they were formed. Many of the basic substances used are biologically active and

in suitable concentration induce toxic reactions culminating in the death of the experimental animal. We found that pretreatment of the animal with heparin markedly enhanced its tolerance to these drugs. For instance, administration of 100 μ g of heparin protected all of the mice challenged with a dose of 48/80 that killed all of the saline-treated controls. Similar protection was obtained against polymyxin, neomycin and stilbamidine. Conversely, the prolonged coagulation time of the blood of heparinized mice could be markedly reduced by the injection of many of these toxic substances.

The observed tolerance of the heparin-treated mice appears to depend initially on the interaction of this acidic mucopolysaccharide with the toxic basic substance. However, the range in complexity of these toxic agents, from a synthetic phenylalkylamine to a naturally occurring polypeptide, makes it unlikely that a specific molecular structure of the toxic agent is required for its fixation by heparin.

Role of the mast cell and fibroblast in micellophagosis

Our observations would suggest that the mast cell may originate in the tissue by a mechanism similar to that known for the granulation of fibroblasts. The mast cell is known to contain relatively large amounts of biologically active substances, heparin and histamine, which appear to be stored in the granules of the cell. Since various kinds of injury to the tissue can cause the degranulation of mast cells and shedding of granules into the surrounding ground substance, we have been interested in determining the fate of this histamine- and heparin-containing material.

Fifteen minutes to an hour after release of the granules, granules staining similarly can be seen in the cytoplasm of surrounding fibroblasts, suggesting that the fibroblasts are able to ingest the shed granules of the mast cells. When granules of mast cells isolated from the subcutaneous connective tissue of mice were injected subcutaneously into other mice, many of the local fibroblasts were subsequently found to have their cytoplasm filled with this granular material. Similar experiments employing isolated S^3 labeled granules of mast cells obtained from mice previously treated with radioactive sodium sulfate confirmed our hypothesis that the granules in the fibroblasts were those shed from the mast cell.

Ingested granules appeared to dissolve or be metabolized within the cytoplasm of the fibroblast, implicating the fibroblast as a participant in the disposal of histamine-containing material as well as in the metabolism of mucopolysaccharides of tissue. In the Arthus phenomenon, the reaction involving the mast cell is more marked and appears to overwhelm the capacity of the local fibroblast to ingest the numerous shed granules.

Discussion

DR EHRICH There have been recent reports indicating that 48/80 releases histamine from the heparin histamine complex suggesting that this material is a histamine liberator. As stilbamidine, adrenocorticotropin et cetera release toluidine blue I wonder whether we can assume that they are also histamine liberators?

Also I should like to ask what you think is the metabolic significance of the uptake of mucopolysaccharides by fibroblasts? Where are the mucopolysaccharides produced? Do you believe it possible that the fibroblasts take up mucopolysaccharides and convert them into hyaluronic acid?

DR HIGGINBOTHAM I think there are degrees of histamine release and 48/80, stilbamidine and polymyxin are histamine releasers both in vivo and in vitro while other substances may be active in vitro only. It seems quite possible that the release occurs by replacement since the releasers combine quite strongly with heparin. How the binding of histamine by heparin comes about is a subject for future work.

The question concerning the turnover of the mucopolysaccharides is difficult to answer. We might assume that fibroblasts are involved in production of hyaluronic acid and other mucopolysaccharides. We made radioautographs of loose connective tissue at various stages after injection of radiosulfate into animals. Radiosulfate has been thought to enter heparin in an exchange reaction with the sulfate of the heparin molecule. However as early as six hours after injection we found a portion of the sulfate fixed in the ground substance and the amount steadily decreased during the next 48 hours. Meanwhile radioactivity in the mast cell increased suggesting the possibility that heparin was being synthesized outside of the mast cell and was then accumulated in it.

Alterations of Fibrinogen and Related Proteins during Mesenchymal Disease

DR RICHARD T. SMITH Most of our studies have been concerned with the origin and nature of the fibrinoid deposits, such as

may be seen in and around the blood vessels in systemic lupus erythematosus in the rheumatic heart and in the connective tissue in rheumatoid arthritis and related disorders. Both clinical and experimental data indicate that this material is composed chiefly of fibrin or a fibrin like protein and a mucopolysaccharide. The generalized Shwartzman reaction provided an experimental model for studying some aspects of the origin and composition of fibrinoid deposits in experimental animals.

Demonstration of reversibly cold insoluble fraction

During attempts to find a circulating precursor of fibrinoid in rabbits given intravenous bacterial endotoxin a component of heparinized plasma that precipitated on standing at 4°C was observed. This material was termed a heparin precipitable fraction since it was reversibly insoluble only in the presence of heparin or related acid mucopolysaccharides. This fraction like fibrinogen disappeared from the circulation with the deposition of fibrinoid material in the kidneys, spleen and heart. Heparinized plasma taken from children with acute rheumatic fever was also found to show a heavy protein precipitate after chilling similar in appearance to that observed in animals which also disappeared when the plasma was warmed.

Physicochemical properties of the heparin precipitable fraction

Material employed for studying the physicochemical properties of the fraction was the twice washed cold precipitate from the heparinized plasma of an acutely ill patient. It was found that heparin, chitin sulfate, polymannuronic acid sulfate and certain dextran sulfates were equally effective in producing cold precipitation in the plasma of acutely ill patients in contrast to calcium binding anticoagulants. Maximal precipitation occurred with the concentration of heparin at 0.1 mg/ml. Increasing ionic strength between 0.02 and 0.5 increased the solubility of the fraction as did increasing pH within the range of 6.7-7.4. Calcium or magnesium ions were required for maximal precipitation.

Several pieces of evidence indicate the close relationship of this fraction to fibrinogen: 1) its absence from heparinized serum; 2) electrophoretic motility similar to that of fibrinogen; 3) 80% of the fraction had sedimentation characteristics in the ultracentrifuge similar to those of fibrinogen; 4) the fraction was 50-80% clottable by thrombin. The fraction differs from pure fibrinogen however in solubility when combined with heparin, marked enhancement of rouleau formation and sedimentation of erythrocytes, only partial clottability and the presence up to 30% of a high molecular weight component.

We are entertaining the working hypothesis that the fraction represents a combination or mixture of fibrinogen and an intermediate polymer of fibrinogen perhaps partially denatured which has an affinity for acid mucopolysaccharides and as a consequence of combination with the e substances has the altered physicochemical properties we have described

Concentration of heparin precipitable fraction in serum

Table 3 presents data on the amount of heparin precipitable fraction found in the plasma of normal individuals of various ages and table 4 summarizes the findings in various disease states. High concentrations of the heparin precipitable fraction occur in the plasma of humans with acute disease whether due to infections or inflammatory or mesenchymal disease. When the patient is severely ill with any of these disorders the concentration often equals or exceeds that of clottable protein. The high values return to normal after successful treatment or spontaneous remission of the disease.

We conclude from such studies that the occurrence of large amounts of heparin precipitable fraction in the plasma of humans is another of the so called acute phase phenomena. The significance of this fraction is not understood. However the occurrence in the circulation during acute disease of a protein with an unusual affinity for acid mucopolysaccharides, suggests a possible function in handling the mucopolysaccharides that result from tissue destruction. The suggestion that the heparin precipitable fraction in humans is a fibrinoid precursor is as yet unsubstantiated but deserves further study.

Discussion

DR DOREMAN: I think most chemists who work with the acid mucopolysaccharides would object to saying that because a material is Schiff positive it is an acid mucopolysaccharide. Some lipids may also be Schiff positive.

DR STETSON: If some of the heparin precipitable protein is non-clottable, why is none found in the serum?

DR SMITH: A likely explanation of the absence of the fraction from serum is that in the clotting process of whole blood fibrinogen absorbs the heparin precipitable fraction of the plasma. However it is entirely conceivable that the antithrombotic effect of heparin itself might influence clottability and give falsely low values in our determinations.

Table 3

Concentrations of heparin precipitable fraction in plasma of normal individuals

Age (y)	N (nb)	M (I)			F (m)		
		No	M (σ)	SD ±	N	M	SD ±
0-15	139	69	0.101	0.013	0	0.104	0.012
16-45	19	20	0.179	0.049	99	0.111	0.009
45-75	188	80	0.111	0.051	103	0.13	0.053

Table 4

Relative concentrations of heparin precipitable fraction in the plasma in various conditions

U y hgh thy hgh th fh og	A ute ur ary t infect n ()
U tre l umat lex (10)	Hed k as d a (3)
U t e l uato l arshne ("0)	U m n t d mal gnancy (4)
U te l a ternal m n n i	P egnan y n t ularly th
pt m n (1?)	toem a (1.00)
A l tregt o ol	
U l ryn t (6)	
U thy hgh with t d fh g	U nt a (4)
Ch on o th umato l a thrt a (5)	U nt eat f conv lesc nt heu
(h n) inf t n of the lung	mat c fevry (6)
u nu a (0)	
U thy with m l l at	
f mmon ld (3)	U pat t s ()
Mump (3)	
U thy t w	
U whom infant (0)	Car l ac f ilu e (3)
Ad need l er d ease (5)	N pl r s (13)
P lvarteris nodosa ()	

() indicate number of patients studied in life

DR S. VAN CREVELD: We know that a heparin neutralizing factor is present in platelets. In plasma if the platelets remain intact the factor is not present.

DR SMITH: We have always been concerned about the possibility of an effect exerted by some factor in platelets. As a consequence we attempt to minimize the platelet effect by appropriate high speed centrifugation as soon as we obtain the blood from the patient.

Behavior of Proteolytic Enzyme Inhibitor in Serum during Inflammatory States

DR. FREDERICK C. MOLL. In the process of inflammation the role of the proteolytic enzymes has not been defined but fibrinolysin and the proteases of leukocytes are thought to contribute to the dissolution of tissue. In the allergic response *in vitro* the liberation of proteolytic enzymes precedes the elaboration of histamine and heparin. Serum however contains inhibitory substances that inactivate the leukoproteases plasmin, trypsin and chymotrypsin. These inhibitory substances may be of importance in the dynamics of inflammation.

The clinical studies of the proteolytic enzyme inhibitor in serum have extended over more than 50 years yet until recently it was known only that the concentration of the inhibitor in the serum is increased in pathologic conditions that are associated with destruction of tissue and the fluctuation in concentration parallels fairly well the edimentation rate. In chronic inflammatory diseases the concentration is elevated at first and returns to normal as the activity of the disease state subsides.

It has recently been demonstrated that the larger fraction of the inhibitor which inhibits trypsin travel electrophoretically with the alpha globulin and a smaller fraction effective against plasmin as well as trypsin travels with the alpha₂ globulin and accounts for only about 10% of the total trypsin inhibitory power of serum.

The method we employed for titration of the inhibitor consists of measurement of the inactivation of trypsin by serum in a casein substrate at 37°C and pH 7.6 the area in which trypsin is most active. The method used was standardized against one employing the inhibitor obtained from soybeans.

Experimental studies in concentration of proteolytic enzyme inhibitor

As may be seen in figure 4 normal monkeys show no significant variation in the level of the serum inhibitor from day to day. With the inflammation produced by a turpentine abscess there is a prompt

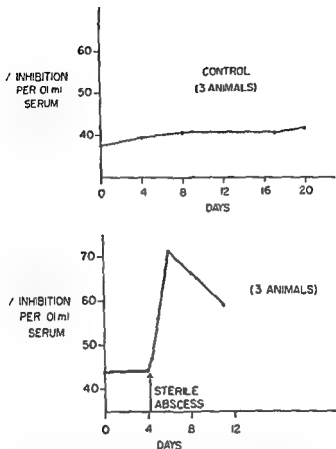


Figure 4 Concentration of proteolytic enzyme inhibitor in serum of monkeys under various experimental conditions (Upper) Normal control animals. Note the constancy of the concentration from day 4 to day 20 (Lower) After production of sterile abscess by injection of sterile abscess there is a prompt increase in concentration. Data from Garg and colleagues, The Journal of Experimental Medicine

rise in the concentration of inhibitor in the serum. This rise has been thought to be due to the accompanying destruction of tissue.

When the entire body was irradiated to produce destruction of tissue there was a prompt and extensive decrease in the total leukocyte count but the concentration of inhibitor in the serum increased only slightly (figure 5 upper). On subsequent production

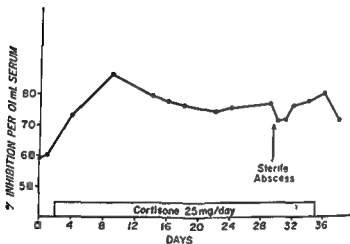
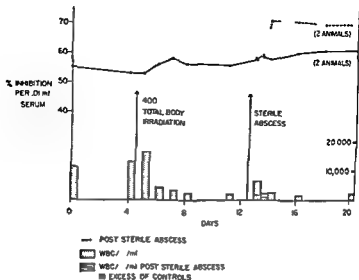


Figure 5 Concentrations of proteolytic enzyme inhibitor in serum of monkeys under various experimental conditions. (Upper) Irradiation of the entire body resulted in a slight but significant increase in the concentration of the inhibitor in the serum but subsequent production of a sterile abscess in two of the animals was accompanied by definite increase. (Lower) The administration of cortisone resulted in a prompt and prolonged elevation of the concentration of inhibitor in the serum and no significant increase in the concentration occurred after the subsequent injection of turpentine. Data from original article in *The Journal of Experimental Medicine*.

of a sterile abscess by injection of turpentine a prompt rise accompanied the inflammatory reaction. When irradiated animals were subjected to electrocautery producing a lesion comparable in size to the inflammatory reaction of the turpentine abscess only a light rise in concentration of the inhibitor occurred.

In humans injection of adrenocorticotropin and exposure to cold temperatures have been shown to result in elevation of the concentration of trypsin inhibitor in the serum. The effect of administration of cortisone was therefore studied in a group of animals and a prompt rise of the inhibitor was noted (figure 5 lower).

The mechanism through which the inhibitory activity is increased by cortisone is not known. No source of the inhibitor in tissue can be demonstrated despite our attempts at assays for the inhibitor in all organs including the sites of inflammation produced by the abscess.

On the twenty eighth day of administration of cortisone an abscess was again produced by injection of turpentine. There was no significant change in concentration of inhibitor in the serum (figure 5 lower). The failure to respond to the inflammatory stimuli during administration of cortisone suggests that the animal failed to elaborate more inhibitor by the usual pathways because of suppression of adrenal activity.

Physiologic effects of isolated proteolytic enzyme inhibitor

The partially purified proteolytic enzyme inhibitor was demonstrated by paper electrophoresis to be primarily an alpha globulin and to contain little if any alpha globulin. The inhibitor was nearly completely destroyed by heat at 56°C for one hour, was unstable at room temperature at pH 3.0 but relatively stable at higher values of pH. The partially purified material was inactive against plasmin which would be anticipated since only the alpha₁ inhibitor is effective against plasmin.

In further studies of this inhibitor in relation to the inflammatory process, guinea pigs were sensitized with egg albumin. Various sites of the skin were then prepared for study by injection of the partially purified inhibitor. The inhibitor injected in some of the sites had been inactivated by heat, while that injected into other sites had not been inactivated. Albumin was then injected into each one of these areas and partial suppression of the inflammatory reaction was observed in the sites of injection of the inhibitor that had not been subjected to heat.

Despite the experimental studies, the clinical significance of the trypsin inhibitor is certainly not clear. It is true the concentration of inhibitor in the serum is elevated in mesenchymal diseases

and returns to normal during remissions of the disease. However with the hyaluronidase inhibitor administration of cortisone appears to raise the concentration of the inhibitor but, when the concentration is high as in rheumatic fever treatment with cortisone is associated with a decrease in concentration and with a subsidence of the inflammatory reaction.

I have postulated that these bizarre and contradictory findings represent changes in the utilization of the inhibitor by the action of cortisone in the presence of large amounts of proteolytic enzymes produced by inflammatory states.

Metabolism of Porphyrins in Rheumatic Fever and Other Mesenchymal Diseases

DR ROBERT A. ALDRICH: Porphyrins are present in physiologically active forms in every living mammalian cell and are involved in the regulation of human physiologic processes. Their elaboration is not so far as we know under the direct control of any of the glands of internal secretion.

Every physiologically active porphyrin in the mammal contains an atom of iron. However both type I and type III isomers of porphyrins are normally found in the free (uncomplexed) form in extremely small amounts in the excreta of mammals. The physiologically active forms of protoporphyrin are hemoglobin, myoglobin, catalase, the cytochromes and peroxidase. In the plant kingdom chlorophyll also is a porphyrin but, instead of iron it contains an atom of magnesium.

Porphyrinuria and porphyria

By custom and common usage we reserve the term porphyria for conditions in which there is an increased rate of excretion of porphyrin in the urine, usually coproporphyrin, and we reserve the term porphyrinuria for certain clinically definable entities, one feature of which is massive urinary excretion of uroporphyrin.

The most common form of human porphyria is characterized by an outpouring of type III porphyrins, both uroporphyrin and coproporphyrin, in the excreta. In this hepatic type of porphyria, the bone marrow is uninvolved and the porphyrins excreted apparently originate in the liver. The nature of the metabolic error

that leads to this enormous excretion of porphyrins is still not completely understood. There may be a metabolic block in the incorporation of iron into protoporphyrin to form heme or there may be another type of biochemical defect located elsewhere in the biosynthetic sequence to protoporphyrin.

Excretion of porphyrins in mesenchymal disease

A significant coproporphyrinuria occurs in acute rheumatic fever, rheumatoid arthritis, polyarteritis nodosa and systemic lupus erythematosus. More than 90% of the coproporphyrin excreted has the type III isomeric configuration.

We have never found in acute rheumatic fever any evidence of a disturbance in the content of free coproporphyrin or free protoporphyrin in the erythrocytes, but I would like to emphasize that most of our studies have been made during the first two or three weeks of the disease. The normal content of these porphyrins in the erythrocytes early in rheumatic fever does not preclude the possibility that metabolism of porphyrin in the bone marrow is abnormal. Most erythrocytes have a life span of 100 days or more, and those cells still in the bone marrow early in the disease may have an increased content of coproporphyrin or protoporphyrin in the free form. Quantitative assay of the amount of free porphyrins in bone marrow has been impossible to interpret.

Our studies of the rate of excretion of coproporphyrins in the urine of patients with mesenchymal diseases have provided interesting sidelights. Despite reports to the contrary, we have never seen administration of salicylates affect the rate of urinary excretion of coproporphyrins unless the salicylates were given in doses sufficient to produce toxic symptoms. In one patient studied at the time of development of erythema marginatum, a manifestation believed by some to indicate increased activity of rheumatic fever, there seemed to be a definite increase in the urinary excretion of coproporphyrin.

Figure 11 presents data obtained in a child whom we had the good fortune to study prior to development of manifestations of rheumatic fever. Coproporphyrinuria was extreme prior to the onset of symptoms of acute rheumatic fever.

Synthesis of heme

In studies of the synthesis of heme we have obtained information that may help to explain the significance of the coproporphyrinuria seen in mesenchymal disease. The manner in which glycine and succinic acid condense to form alpha-amino-beta-ketoadipic acid and its subsequent conversion to delta-aminolevulinic acid are now

quite well understood. We know also that two molecules of delta aminolevulinic acid condense to form porphobilinogen. Porphobilinogen or delta aminolevulinic acid can be converted to heme with ease by a variety of extracts from mammalian and plant cells. Our knowledge of the way in which iron is incorporated into the protoporphyrin to form heme is meager.

Using a system of lysed duck erythrocytes for studying the synthesis of heme Neve found that with the addition of glycine succinate and radioiron radioactive heme was formed. Addition of

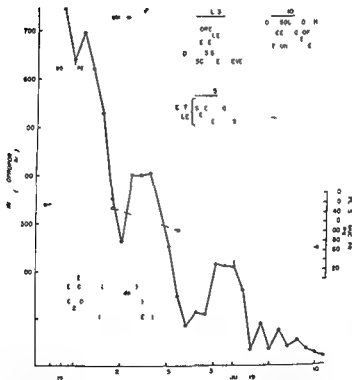


Figure 6 Rate of excretion of coproporphyrin in the urine and the concentration of mucoprotein in the plasma of a child prior to and after the development of acute rheumatic fever. During and immediately after the attack of scarlet fever which preceded the rheumatic fever in this child the rate of excretion of coproporphyrin in the urine was high but decreased to normal with the development of manifestations of acute rheumatic fever. Reprinted from Pediatrics 15:557 (May) 1955.

highly purified protoporphyrin did not result in any significant increase in the amount of radioheme formed indicating that protoporphyrin itself may not be in the direct pathway of heme synthesis. However, he found that when reduced uroporphyrin III was added to the same system there was a tremendous increase in the amount of radioactivity in the heme. Thus reduced uroporphyrin III may lie in the direct pathway for synthesis of heme.

The use of reduced protoporphyrin, uroporphyrin I, reduced uroporphyrin I or uroporphyrin III (not reduced) in this system produced practically no radioheme. Reduced coproporphyrin III seemed to activate the system but not nearly as efficiently as did reduced uroporphyrin III. Additional isotope dilution studies gave confirmatory evidence that reduced uroporphyrin III is in the direct pathway of synthesis of heme.

These data suggest that reduced uroporphyrin III and possibly reduced coproporphyrin III might be important intermediates in the biosynthesis of heme. Whether reduced coproporphyrin or reduced uroporphyrin actually accept iron I do not know. Perhaps reduced uroporphyrin III combines with the iron atom and other enzymes decarboxylate the side chains of uroporphyrin to produce coproporphyrin. Then if two carboxyl groups were changed to vinyl groups, iron protoporphyrin (i.e. heme) would result.

We know that much synthesis of heme occurs in the cells of the liver and in porphyria the amount of synthesis in this site can be considerable. It is certainly conceivable that coproporphyrinuria could result from a disorder in hepatic synthesis of heme in mesenchymal diseases. It is also possible although as yet we have no evidence, that an abnormality in metabolism of porphyrins in mesenchymal diseases is localized in the bone marrow.

Discussion

CHAIRMAN GEORGE E. CARTWRIGHT: We have obtained evidence in our laboratory that protoporphyrin is an intermediate in the synthesis of heme. Our experimental conditions are a little different from those of Neve and this probably explains the differences in the results.

I would seriously doubt that the presence of coproporphyrinuria in itself indicates a serious error in metabolism of porphyrins. Coproporphyrinuria is a very common finding as you know in a great many conditions.

I was very much interested to hear that the content of free protoporphyrin and coproporphyrin in erythrocytes was found to be normal in acute rheumatic fever. Our own studies are in agreement

with those of Aldrich for acute cases. However we have found rather striking increases in the content of free protoporphyrin in the erythrocytes of children that have been sick for 60 days or more.

DR R. A. ALDRICH: It is certainly true that protoporphyrin will take up iron to form heme in vitro in the system used in the studies by Cartwright's group. We have used the same phosphate buffer system and confirmed the results.

Whether the conditions we employed for synthesis of heme from reduced uroporphyrin III are more nearly physiologic than those employing the phosphate buffer remains to be settled. It seems significant to us, however, that protoporphyrin is not converted to heme under these conditions. I believe that the evidence favors a physiologic role for reduced uroporphyrin III and probably for reduced coproporphyrin III.

Anemia in Mesenchymal Disease

DR M. EUGENE LAHEY: Anemia accompanying mesenchymal diseases, whether occurring in children or adults, may be attributable to various causes such as concomitant deficiency of iron or loss of blood as occurs in the patient with anaphylactoid purpura. This discussion, however, will not be concerned with anemia of such obvious cause but with the anemia of uncertain etiology that rather commonly complicates some of the mesenchymal diseases.

What factors influence the development or the severity of anemia in these disorders have not been completely elucidated. The following, however, appear to play a role. The severity of the systemic reaction to the underlying disease seems to be important, as indicated by the frequent occurrence of anemia during recrudescences in rheumatic fever and the restoration of hemoglobin values to normal during convalescence. The duration of the disease would seem to be important. In systemic lupus erythematosus, for example, patients not anemic at the outset often become so as the disease persists or progresses. That the age of the patient is also important is suggested by the greater frequency of anemia in children with polyarteritis nodosa and rheumatoid arthritis than in adults with the same diseases. Whether other factors, such as heredity, previous nutritional status, or emotional factors, play a role in the development of anemia is at present uncertain.

Evidence for decreased rate of erythropoiesis

The concept that anemia arises primarily as a result of decreased erythropoiesis rests largely on evidence from the study of patients with rheumatoid arthritis. This evidence includes the insidious onset of anemia, reticulocytopenia, the absence of classic signs of hemolysis, the diminution in number of erythroid elements and maturation arrest of normoblasts in the marrow. In rheumatoid arthritis alterations in the metabolism of iron, copper and porphyrins are strikingly similar to those in the anemia associated with chronic infections. In the latter instance anemia is due primarily to decreased marrow function.

Evidence for increased rate of hemolysis

There are also observations that support the concept that excessive hemolysis plays a role. Convincing evidence has been presented that severe hemolytic anemia may be the first manifestation of systemic lupus erythematosus, often antedating other manifestation of the disease by months or even years. Such overt hemolytic anemia has occurred in 5 to 20% of adults and in almost one third of the small number of children studied. The direct Coombs test is often positive even in the absence of obvious hemolysis. This test, however, is seldom positive in other rheumatic diseases.

Hemolytic anemia has also been noted in a few patients with polyarteritis nodosa and in children with anaphylactoid purpura. The signs of hemolysis have consisted of sudden onset of anemia, reticulocytosis, normoblastic hyperplasia of the marrow, anisocytosis and poikilocytosis of mature erythrocytes and evidence of excessive excretion of products of the degradation of hemoglobin.

The introduction of differential agglutination and isotopic techniques has made it possible to inquire more thoroughly into the role of accelerated hemolysis in the anemia of various disease states. It is now evident that though overt hemolysis may be uncommon, minor degrees of hemolysis are not infrequent in at least some types of rheumatic disease. In rheumatic fever the survival of normal cells has been shown to be reduced by 15 to 20%. Similarly, in rheumatoid arthritis, the life span of the erythrocytes may average only 70 to 100 days instead of the usual 110 to 120 days. These observations have been made after the administration of normal cells to patients with these disorders. When cells from the patients, however, are given to normal recipients, the survival time is normal, indicating that the hemolysis is a result of extracorporeal rather than intrinsic or intracorporeal defects.

Possible causes of decreased rate of erythropoiesis and increased rate of hemolysis

Certain hypotheses can be offered to explain the occurrence of either decreased function of the marrow or accelerated destruction of erythrocytes as a cause of anemia in mesenchymal disease. Some evidence suggests that the primary cause of lessened erythropoiesis lies in defective synthesis of hemoglobin. The normal or increased content of protoporphyrin in the erythrocytes suggests that early steps in the synthesis of hemoglobin are not affected. It may be that the defect exists at the point where iron is incorporated into the porphyrin molecule to form heme or that the fault lies in the fabrication of globin. Since the metabolism of other proteins is seriously affected in these disorders, it is not unreasonable to suspect that the erythrocyte is affected simply because there are other more important uses for iron, protein and other constituents during the active phase of the disease.

It has been postulated that a variety of stimuli—chemical, bacterial, viral, et cetera—may provoke changes in the erythrocyte so that it becomes antigenic with ensuing autoimmune hemolytic disease. Just as some individuals form antibodies to erythrocytes with remarkable facility after transfusions of blood, some may also form antibodies with the same facility in response to whatever stimulus provokes the mesenchymal disorders. Such an altered immunologic reaction may account for the hemolytic anemia and the positive Coombs test noted especially in patients with systemic lupus erythematosus. Antibodies may have an adverse effect not only on mature erythrocytes but also on reticulocytes and even on their nucleated precursors in the marrow.

Considerations regarding attempts at correction of the anemia

The anemia of mesenchymal diseases is usually mild and the welfare of the patient does not seem to be jeopardized by the degree of anemia usually encountered, nor does correction of the anemia hasten recovery. Vigorous therapy directed against the anemia per se is therefore seldom warranted, especially when we consider the greater frequency of reactions to transfusions in these patients. In occasional instances of brisk hemolysis, such as occur not infrequently in systemic lupus erythematosus, transfusions of blood may be life saving.

Anemia usually subsides in those patients successfully treated with adrenocorticotropin, cortisone or a related substance. It is probable that improvement comes from the effect of these agents on

the underlying disease. In patients with systemic lupus erythematosus or rheumatoid arthritis complicated by hemolytic anemia or pancytopenia splenectomy has occasionally been performed but usually with only transient hematologic improvement. The scattered reports that administration of iron is of value in alleviating the anemia of rheumatoid arthritis are probably based on improvement coinciding with spontaneous recovery from the underlying disease or to correction of a coexisting deficiency of iron. Many other therapeutic agents have been tried for the anemia of mesenchymal diseases but their value has not been substantiated and definite risk may attend their use.

Hormonal Alterations in Mesenchymal Disease

Biosynthesis of Adrenal Steroids

DR LEO T. SAMUELS: The more important hormones of the adrenal gland are 17-hydroxycorticosterone (cortisol or compound F), corticosterone, aldosterone, 11 β -hydroxyandrostenedione, androstenedione, and probably estrone. All have a four-ring structure, the cyclopentenophenanthrene nucleus.

Synthesis of cholesterol

The importance of cholesterol in the formation of the adrenal steroids was recognized first by observation of the decrease in content of cholesterol in the adrenal gland after stress. The isolation of radioactive adrenal steroids after perfusion of radioactive cholesterol through adrenal glands demonstrated that cholesterol could be a precursor of the adrenal hormones.

Cholesterol can be synthesized also from acetate in the adrenal gland, and it seemed possible that the acetate incorporated in adrenal hormones might have been incorporated in cholesterol as an intermediate.

As indicated in figures 7 and 8, the steps in the synthesis of cholesterol from acetate appear to involve the following intermediates: acetyl-CoA, acetoacetate, and β -hydroxy- β -methylglutaric acid. The last substance, if labeled with radioactive isotope and introduced into a homogenate of liver or adrenal gland, will give rise to radioactive squalene and cholesterol. Oxygen is necessary in the reaction, although the overall result is a reduction. Triphosphopyridine nucleotide in reduced form is also necessary.

Synthesis of various hormones of the adrenal cortex

Using the supernatant fluid from a homogenate of the adrenal glands under controlled conditions of pH and in the presence of triphosphopyridine nucleotide, Guerin has found that cholesterol is converted rapidly to pregnen-3 β -ol-20-one, a compound closely related to the adrenal hormones and found in both the adrenal glands and testes.

Heard has been able to synthesize the adrenal steroids from acetate in a cell free system. Previously such synthesis had been done with systems containing organized cells. Utilizing radioactive labeled acetate in this cell free system cortisol with high radioactivity was produced, but no radioactive cholesterol as though under these circumstances there had been no conversion to cholesterol, and all the acetate had gone by some other route into the adrenal hormones. These and other findings suggest that cholesterol may not be an indispensable intermediate in the formation of adrenal hormones.

Figure 9 indicates the general reactions that are probably involved in the synthesis of progesterone, 17 hydroxyprogesterone, desoxycorticosterone, 17 hydroxycorticosterone (cortisol), corticosterone, androstenedione and 11 beta hydroxyandrostenedione. Evidence has been presented by Hechter that these reactions must occur in sequence. Perfusion of the adrenal gland with 17 hydroxyprogesterone resulted in production of substance S which includes the 21 hydroxy group and of cortisol but not of corticosterone. When desoxycorticosterone was perfused corticosterone but not cortisol was obtained. Similar results suggesting that these reactions must take place in sequence were obtained from our studies in vitro. It has been shown more recently that this sequence is not obligatory but under the conditions usually found in normal cells hydroxylation ordinarily does occur in this sequential fashion.

It seems that the presence of triphosphopyridine nucleotide in the reduced form is necessary whenever replacement of hydrogen with hydroxyl must take place on a $-\text{CH}-$ or $>\text{CH}-$ group. If the reaction is a simple dehydrogenation with the conversion of an hydroxyl group to a ketone or the formation of a double bond then oxidized diphosphopyridine nucleotide is needed on a CH_2 group. These are the major coenzymes in steroid biosynthesis.

Aberrations in the various enzyme systems may result in formation of androgenic or estrogenic tumors or of tumors that might simply produce excess amounts of corticosterone, cortisol or aldosterone. By knowing these steps in biosynthesis we can look for compounds that might compete with the usual substrates of abnormally functioning enzymes, and thus stop the production of undesirable hormones. New compounds have been discovered that do seem to compete in this fashion to some extent.

Pro- and Antiphlogistic Steroids

DR THOMAS F. DOUGHERTY: The steroid hormones may be divided into those which tend to diminish the degree of inflammatory

response called antiphlogistic and those which tend to increase the inflammatory response called profllogistic

Theoretic considerations

Selye has suggested that there is a balance between the amounts of anti and profllogistic steroid hormones secreted by the adrenal cortex and that the defect responsible for the production of mesenchymal diseases may be an alteration in adrenal cortical secretion. He believes that an increased output of profllogistic hormones may counterbalance the anti-inflammatory effects of such compounds as cortisol (hydrocortisone).

By this hypothesis desoxycorticosterone is the prototype of the profllogistic hormones. The naturally secreted profllogistic hormone is aldosterone which is approximately as profllogistically potent as desoxycorticosterone. Growth hormone may be the trophic hormone for secretion of aldosterone.

Our studies indicate that desoxycorticosterone markedly reduced the resistance to anaphylactic shock and to local inflammation induced by allergic reactions. When desoxycorticosterone is given concurrently the anti-inflammatory and antianaphylactic capacities of cortisone are reduced. However we have no definite evidence that the adrenal cortex in mesenchymal diseases is functionally altered in such a way that aldosterone predominates in the secretion of the gland and we have no convincing evidence that growth hormone is the trophic hormone for aldosterone.

Technique of study

Subcutaneous tissue is spread on a slide so that it is practically one cell thick and it is then possible to count the number of cells in an area 1 cm on a side and to calculate the number of cells in 1 cm² of tissue. The method of counting is analogous to that of the ordinary blood count.

Figure 10 demonstrates the appearance of a spread of tissue from an area of inflammation produced by injection of a standard amount of gelatin a substance evoking a mild inflammation of consistent appearance. Also shown is the appearance of tissue from a similarly treated area of an animal that had received cortisol in addition to the gelatin. The anti-inflammatory effect of cortisol is apparent.

By this technique it was shown that cortisol is a more potent anti-inflammatory steroid than cortisone. Study of analogs of the potent hormones makes it possible to ascertain the structural relationship that determines the degree of potency provided by an anti-inflammatory hormone.

Characteristics of antiphlogistic hormones

We find that the unsaturation of the A ring is absolutely essential for anti inflammatory effect. The presence of the 17 hydroxy group is also essential and the presence of an hydroxy or oxygen



Figure 10 Appearance of a spread of tissue prepared from an area of inflammation produced by injection of gelatin (upper) and of tissue from a similar area of an animal that received hydrocortisone in addition to the gelatin (lower). The absence of polymorphonuclear cells and of other evidences of inflammation is apparent in the tissue from the animal that received hydrocortisone.

group at the 11 position produces marked potentiation of the anti-inflammatory effect. We hope to study the whole series of hormones that apparently occur naturally in the adrenal cortex.

Some years ago we established the fact that the anti-inflammatory effect of cortisol is exerted at the site of its presence and that it is not related to or dependent upon circulation through the liver. Thus cortisol itself rather than one of its conversion products appears to give the anti-inflammatory effect.

Radioactive (4 C^{14}) cortisol was given intravenously to adrenal-ectomized animals at the same time that an inflammation was produced by injection of gelatin into one flank, the opposite flank acting as control noninflamed tissue. The concentration of hormone in noninflamed tissue paralleled the pattern of that in inflamed tissue although the inflamed tissue accumulated more of the radioactive hormone.

In studies of the rate of conversion of cortisol to glucuronide by the liver we demonstrated that the concentrations of conjugated and nonconjugated hormone are approximately the same in the blood as in the area of inflammation. Because conjugation of cortisol occurs only in the liver, the similarity of concentrations in blood and in inflamed tissue indicates ready transit of the hormone between these two areas.

In making radioautographs after subcutaneous administration of radioactive cortisol we have learned that the radioactive substance tends to localize intracellularly in the fibroblast and fat cell but that the duration of its presence in these cells is rather short compared with the prolonged anti-inflammatory effect it produces. Therefore we suspect that it is an alteration at the fibroblastic cell level which the hormone produces in the tissue rather than its continued presence that is essential for anti-inflammatory activity.

Discussion

DR DORFMAN: Schiller and I have been trying for a long time to study directly the effect of cortisol on synthesis of polysaccharides. When we pretreated animals with cortisol there was a marked inhibition of synthesis of both hyaluronic acid and chondroitin sulfate in the skin. Maximum inhibition did not occur for about five days.

DR DOUGHERTY: Layton also found a delay between the administration of cortisone and appearance of inhibition in formation of ground substance.

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The hormone labeled isotopically disappears from the organism within about 48 hours. It cannot be identified in a localized site within a much shorter period of time.

It is likely that the many actions of cortisol are the result of its effects on many different systems. It may not have to be present in excess when its activity is demonstrated by any physiologic test. It does not surprise me that inhibition of synthesis of polysaccharides does not occur for some time after the cortisol is administered.

DR DAVID GLICK: Would you comment on the claim that cortisol causes destruction or disruption of the mast cells?

DR DOUGHERTY: I do not believe we can say that there is any specific effect of cortisol in disruption of mast cells.

Quantitative Histochemical Studies of the Adrenal Gland in Various Physiologic States

DR DAVID GLICK: We chose the quantitative histochemical approach in an attempt to obtain information about the localization in the adrenal gland of some of the important biochemical processes important in its functioning. We worked out a method of killing animals suddenly with what we believed would be minimal stress. Within 30 seconds after an animal had been peacefully sitting in his cage with no anticipation that anything would happen, his adrenals were frozen in dry ice. The adrenals were then not permitted to thaw until the final chemical analysis or histologic study was made.

A cylinder of tissue was drilled from the frozen adrenal and mounted on a rotary microtome. Sections were taken through all the zones of the cortex and through the medulla. Individual sections were used for chemical analysis and adjacent ones for histologic identification so that a fairly good degree of correlation could be achieved between histologic and chemical findings. All of the analyses were performed by colorimetric or spectrophotometric methods, some times on volumes of fluid as small as 0.005 ml.

Studies of ascorbic acid and cholesterol in the adrenal cortex

Ascorbic acid is lost suddenly from the adrenal gland when the organ is stimulated either hormonally or by stress. It represented

graphically in figure 11 are the results of our studies to determine in what zones of the adrenal cortex the loss occurs and the actual amounts of ascorbic acid lost as the result of a given treatment. When monkeys were given a single subcutaneous injection of adrenocorticotropin three hours before sacrifice a decrease in total concentration of ascorbic acid was observed. If the animals were stressed by a combination of hypoxia and hypothermia there was an even more drastic decrease. When rats were put in death by ether or were

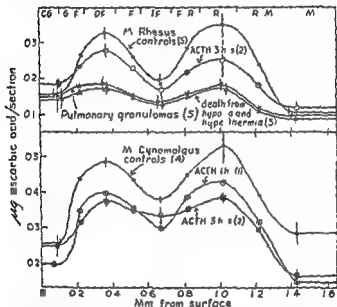


Figure 11 The concentration of ascorbic acid in various portions of the adrenal gland of the rhesus (upper) and cynomolgus (lower) monkeys under conditions of minimal stress (highest curve) and after administration of adrenocorticotropin or production of stress in various ways. The letter along the top of the figure refers to the histological zone of the adrenal gland: C-capsule, G-glomerular, F-fasciculated, OF-outside fasciculated, IF-inner fasciculated, M-medulla. Reprinted from *Endocrinology* 51:66 (June) 1951.

given an injection of adrenocorticotropin one hour before sacrifice the same general effect was obtained. If they were merely held in the hand causing them to struggle a maximum loss of ascorbic acid occurred within two minutes.

Loss of cholesterol also occurs when the adrenal glands are stimulated hormonally or when the animal is subjected to environmental

stress In the unstressed cynomolgus monkey we found that practically all the cholesterol in the adrenal was in the form of ester It was concentrated in the outer fascicular zone and to a lesser degree in the reticular area Three hours after injection of adrenocorticotropin the concentration was markedly decreased On the other hand injection of cortisone produced no effect on the content of cholesterol in any zone

Studies of enzymes that deconjugate hormones

Beta glucuronidase an enzyme present in appreciable amounts in the adrenal is known to liberate free hormone from the conjugated form We calculated the content of beta glucuronidase in the various zones of the adrenal cortex of the rhesus monkey by determining the rate of liberation of phenolphthalein A peak concentration of beta glucuronidase was found in the reticular zone in both the untreated animal and the animal treated with adrenocorticotropin with no real difference in concentration between the two

In this zone the content of protein nitrogen was also elevated We believe that this determination represents a better index of concentration of active metabolizing enzyme than does dry weight or volume of tissue because especially in the adrenal so large a proportion of the tissue is lipid substance

We found remarkable differences in concentrations of beta glucuronidase in the adrenal among animals of different species For example in bovine adrenal there is a definite high concentration in the glomerular zone and less activity in other zones

After hypophysectomy the adrenals of rats undergo atrophy and weigh only one half as much as in control animals Histologically the relative distribution of beta glucuronidase in the adrenal is unchanged The highest concentration is still in the reticular zone but the magnitude is increased remarkably

We speculated that this might mean increased active free hormone liberated from conjugate circulating in the blood and picked up by the adrenal While the need for adrenal hormone continues because of atrophy the organ cannot respond with production of hormone Since hormones are conjugated to sulfate as well as to glucuronic acid deconjugation by phenolsulfatase might also be expected to occur in the adrenal

The concentration of phenolsulfatase was found to be greatest in the fascicular region and no change was produced by prior treatment with desoxycorticosterone acetate adrenocorticotropin or cortisone in the doses we employed Similarly chilling or formation of a sterile abscess by injection of turpentine had no effect on the concentration of phenolsulfatase The activity remained remarkably

constant in spite of very drastic environmental changes. Six days after hypophysectomy at a time when a great deal of atrophy of the adrenal had already occurred instead of an increased concentration as with beta glucuronidase there was actually a decrease in concentration of phenolsulfatase. I do not know why there should be this difference in the effect of hypophysectomy on the level of the two enzymes both of which deconjugate hormones.

Studies of cholinesterase succinic dehydrogenase and coenzyme A

Cholinesterase is concentrated in the medulla of the adrenal gland of the rat there is almost none in the cortex as might be expected since the neural elements in the gland are concentrated in the medulla. After administration of a variety of hormones and production of stress by various means no effect on the concentration of cholinesterase was observed. The atrophy of the adrenal resulting from hypophysectomy also effected no change in concentration of cholinesterase. The concentration of succinic dehydrogenase an enzyme involved in the Krebs cycle was also unaffected by administration of adrenocorticotropin or cortisone.

Deficiency of pantothenic acid results in decreased rate of liberation of adrenal hormones. Coenzyme A a derivative of pantothenic acid is essential for the production of cholesterol from acetate in the adrenal. We found that the peak of distribution of coenzyme A is in the reticular zone. After treatment with adrenocorticotropin or desoxycorticosterone there was little change. There was no change at all six days after hypophysectomy as compared to the sham operated control.

It may be that for certain important enzymes in tissues a remarkably constant concentration is maintained even in spite of pharmacologic influences. Possibly these enzymes are so essential for the function of the tissues that an excess is always supplied.

Response of the Pituitary-Adrenal System to Psychic Stimuli

DR. ELCENE L. BLISS. The work that I am about to report was undertaken in an attempt to learn whether psychologic disturbances in man were associated with alterations in adrenocortical function.

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... the plasma cortisol level, the estimate of per cent ... was made to distinguish ... an emotional disturbance exhibited ...

... were graded as either ... other times, emotional ... in which 0 represented ... All estimates of emotional ... of chemical results.

Study of psychotic patients

... was studied ... ward. Seven ... were found ... in the ... of 13 μ g/100 ml in ...

... the schizophrenic variety ... and whose manifest ... had concentra ... limits ... chronic schizo ... in the blood because ... bizarre ... in schizo ... of 17 by ...

Study of normal subjects

... their ...

... the blood ... the ... and the ... during the period of ...

From other studies it has become evident that the increases in concentration of 17 hydroxycorticosteroids in the plasma during emotional stress are relatively modest in comparison with the effect of other stressful procedures. Most potent of the procedures studied is administration intravenously of adrenocorticotropin.

The trends in our studies were remarkably consistent. Increases in the concentrations of 17 hydroxycorticosteroids in the blood averaged 5 to 7 $\mu\text{g}/100\text{ ml}$ with emotional stress. All values were within the known physiologic limits even in the face of the most disabling

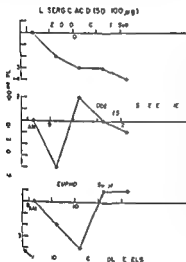


Fig. 12 The effect of adrenocorticotropin (ACTH) on 17-hydroxycorticosteroid levels in plasma and urine in normal subjects. In five individuals who received 100 units of ACTH intravenously, the concentration of 17-hydroxycorticosteroids in the plasma were within normal limits, decreased to the normal diurnal pattern. In the other subject who became quite disturbed during the period of maximum emotional disturbance. Four subjects displayed a typical response which was associated with increase in excretion of 17-hydroxycorticosteroid in the blood. Reprinted from *Psychosomatic Medicine* 18: 65, 1956.

and catastrophic panic. Because of the consistency of the results in a variety of individuals studied under diverse types of emotional stress, it seems likely that acute states of emotional disturbance and heightened anxiety are routinely, although perhaps not invariably, associated with small but significant increases in the concentrations of 17 hydroxycorticosteroids in the blood. Since there is also an increase in the rate of excretion of the 17 hydroxycorticosteroids in the urine, it seems likely that emotional stress is associated with a modest increase in adrenocortical activity.

Influence of central nervous system

It seems probable that the central nervous system influences the anterior pituitary gland via hypothalamic centers. We have tentative indirect evidence of the relationship between the central nervous system and the endocrine glands. When patients receive electroshock

therapy there is an increase with the grand mal seizure of the concentration of 17 hydroxycorticosteroids in the plasma. When these seizures are blocked by suitable anticonvulsive agents so that the muscular movements are inhibited except for a slight twitching of the eyelids the concentration of 17 hydroxycorticosteroids increases nevertheless.

In decerebrate human subjects the concentration of 17 hydroxycorticosteroids in the plasma is consistently greater than in normal subjects.

It seems that electrochemical and psychologic processes arising in the cerebral cortex have an important effect upon the activity of the anterior pituitary gland which in turn causes the adrenal cortex to modify its rate of secretion. Under the impact of acute emotional stress the concentration of 17 hydroxycorticosteroids in the blood will increase. The degree of elevation is usually modest and remains within physiologic limits. Cortical discharge induced by electroshock will also produce elevations in these concentrations even when peripheral convulsive activity is blocked.

Pituitary-Adrenal Function in Mesenchymal Diseases

Evidence of endocrine disturbance in mesenchymal diseases

DR ROBERT S. ELY: The first clinical trials with cortisone were performed in patients with rheumatoid arthritis because clinical experience had suggested the possibility that adrenocortical function has an influence on the course of the disease. Subsequently reports of the beneficial effects of cortisone and adrenocorticotropin in other mesenchymal diseases strongly invited the belief that an altered pituitary-adrenal function was present.

A relationship between mesenchymal diseases and adrenal function was also suggested by studies of certain acute phase reactants, particularly the nonspecific hyaluronidase inhibitor. The concentration of this inhibitor in the plasma is lower than normal in adrenal-ectomized or hypophysectomized animals, and administration of adrenocorticotropin or cortisone produces a rise. Inhibitor concentration is low also in patients with rheumatic fever except during the early acute phase of the disease when it is elevated as it is in other acute illnesses.

Animal experimentation has shown a definite influence exerted by hormones on the function of connective tissue. The mineralocorticoids, or phlogistic corticoids, enhance reactivity of connective tissue whereas the glucocorticoids tend to act in an opposite manner. Despite this it is known that administration of either type of hormone can under experimental conditions induce in animals lesions similar to those of mesenchymal diseases.

17 hydroxycorticosteroids in acute rheumatic fever

We have been interested for some time in evaluating pituitary-adrenal function in children with rheumatic fever and related diseases. Certain abnormalities in pituitary-adrenal function seem to be present although their significance lies beyond interpretation at the present time. In children with early acute rheumatic fever—that is, during the first week of rheumatic activity—the concentration of 17 hydroxycorticosteroids in plasma is approximately twice as great as those of control children. It should be mentioned here that patients with other acute illnesses commonly have concentrations higher than those seen in patients acutely ill with rheumatic fever. In patients who have had active rheumatic fever for at least two weeks the concentration of 17 hydroxycorticosteroids is consistently low. In patients with inactive rheumatic fever the concentration is also low.

Adrenal insufficiency

This low concentration of 17 hydroxycorticosteroids in the plasma is interpreted by us as indicating the presence of adrenal insufficiency. The concentration does not appear to be low because of an increased rate of utilization by tissue, since the rate of disappearance of administered steroid actually is lower than normal.

If the adrenocortical hypofunction were primary rather than secondary to insufficient stimulation by the pituitary, we would expect increased secretion of adrenocorticotropin by the pituitary to be associated with the decreased secretion of steroid by the adrenal gland. In patients with rheumatic fever, except during the early acute phase, the concentration of 17 hydroxycorticosteroids in the plasma is lower than normal despite an elevated concentration of adrenocorticotropin. Since the rate of disappearance of these steroids from the circulation is not increased, these data are interpreted as indicating that the patient with rheumatic fever has primary adrenal insufficiency.

That adrenal insufficiency is relative rather than absolute is indicated by the significant increase in concentration of 17 hydroxycorticosteroids in the plasma after administration of a large test dose of adrenocorticotropin. The nature of the insufficiency remains somewhat obscure. Diminished concentration of 17 hydroxycorticosteroids in the plasma is not reflected in decreased rates of urinary excretion.

therapy there is an increase with the grand mal seizure of the concentration of 17 hydroxycorticosteroids in the plasma. When these seizures are blocked by suitable anticonvulsive agents so that the muscular movements are inhibited except for a slight twitching of the eyelids the concentration of 17 hydroxycorticosteroids increases nevertheless.

In decerebrate human subjects the concentration of 17 hydroxycorticosteroids in the plasma is consistently greater than in normal subjects.

It seems that electrochemical and psychologic processes arising in the cerebral cortex have an important effect upon the activity of the anterior pituitary gland which in turn causes the adrenal cortex to modify its rate of secretion. Under the impact of acute emotional stress the concentration of 17 hydroxycorticosteroids in the blood will increase. The degree of elevation is usually modest and remains within physiologic limits. Cortical discharge induced by electroshock will also produce elevations in these concentrations even when peripheral convulsive activity is blocked.

Pituitary-Adrenal Function in Mesenchymal Diseases

Evidence of endocrine disturbance in mesenchymal diseases

DR ROBERT S. ELY: The first clinical trials with cortisone were performed in patients with rheumatoid arthritis because clinical experience had suggested the possibility that adrenocortical function has an influence on the course of the disease. Subsequently reports of the beneficial effects of cortisone and adrenocorticotropin in other mesenchymal diseases strongly invited the belief that an altered pituitary-adrenal function was present.

A relationship between mesenchymal diseases and adrenal function was also suggested by studies of certain acute phase reactants, particularly the nonspecific hyaluronidase inhibitor. The concentration of this inhibitor in the plasma is lower than normal in adrenalectomized or hypophysectomized animals, and administration of adrenocorticotropin or cortisone produces a rise. Inhibitor concentration is low also in patients with rheumatic fever except during the early acute phase of the disease when it is elevated as it is in other acute illnesses.

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Studies relating to other steroids

The occurrence of a segmental adrenal insufficiency in certain conditions is known. In congenital adrenal hyperplasia the patient produces only small amounts of 17 hydroxycorticosteroids, although he consistently produces an excessive quantity of certain other steroids. A specific deficiency of one of the many enzymes involved in steroidogenesis appears responsible for the abnormal metabolism in this disease. Study of other steroids seemed desirable in children with mesenchymal diseases and it was found that, in active and inactive rheumatic fever and in rheumatoid arthritis, the mean concentration of corticosterone in the plasma is significantly higher than in control subjects.

Responsiveness of the adrenal gland is clearly dependent on normal metabolism of the circulating steroids. The factors involved in steroid metabolism other than conjugation and excretion are not well known. However, one approach to the problem consists of determining the half life of administered steroid. We studied only the rate of removal of steroid in the free form and have no data bearing on the rate of conjugation or of excretion. The mean half life of cortisol or corticosterone in active rheumatic fever and rheumatoid arthritis was significantly greater than in control children of similar age.

Studies of families of patients with rheumatic fever

In considering factors that determine the reaction of the host in the production of mesenchymal diseases one postulate is that rheumatic susceptibility is genetically determined. If a hereditary physiologic or biochemical factor were responsible for this rheumatic susceptibility it might be demonstrable in families of patients. Because of the consistent finding of low concentrations of 17 hydroxycorticosteroids in the plasma of subjects with rheumatic fever we undertook a study of their families.

In table 5 the concentrations of 17 hydroxycorticosteroids and of corticosterone in plasma of parents and siblings of patients with rheumatic fever are shown. The concentration of 17 hydroxycorticosteroids is significantly decreased in parents and siblings, whether these individuals had positive histories of rheumatic fever or not. On the other hand the concentration of corticosterone in the plasma of the siblings is greater than normal paralleling the increased concentration seen in children with rheumatic fever and other mesenchymal diseases. The parents, however, do not show a significant alteration.

The significance of these evidences of abnormal adrenal function and abnormal metabolism of corticosteroids in the pathogenesis of mesenchymal disease is not clear.

Table 5

Concentration of 17 hydroxycorticosteroids and of corticosterone in the plasma of members of families of patients with rheumatic fever

Group	Number of Subjects	Mean Plasma Concentration ($\mu\text{g}/100\text{ml}$)	Standard Error of Mean
17 OHCS			
Control	40	120	± 1.29
Siblings			
+Rheumatic RF	6 ^a	75	± 0.68
-Rheumatic RF	143	69	± 0.16
Parous			
+Rheumatic RF	16	77	± 1.53
-Rheumatic RF	6		± 0.63
Corticosterone			
Controls			
5-17 years	31	30	± 0.30
Adults	71	30	± 0.1
Siblings			
+Rheumatic RF	28	63	± 1.4
-Rheumatic RF	6	66	± 0.91
Parous			
+Rheumatic RF	7	5	± 0.49
-Rheumatic RF	3 ^a	4	± 0.5

In patients with rheumatic fever the relative adrenal insufficiency consisting of a low concentration of 17 hydroxycorticosteroids and elevated concentrations of corticosterone and adrenocorticotropin in the blood raises the vital question: does this pattern of adrenal function precede the first attack of rheumatic fever or is it a result of the attack? Similar findings in the plasma of patients whose rheumatic fever has been inactive for many months suggests that it precedes the rheumatic attack. Even more important, the presence of these same findings in otherwise normal siblings of patients with rheumatic fever is strongly suggestive that altered adrenal function antedates the first attack of rheumatic fever.

Clinical Aspects of Mesenchymal Diseases

Juvenile Rheumatoid Arthritis

DR THOMAS A GOOD This report deals with data concerning 73 cases of juvenile rheumatoid arthritis studied during the last 10 years

Still's triad of findings for the diagnosis of juvenile rheumatoid arthritis consisted of arthritis lymphadenopathy and splenomegaly. Because of the variability of manifestations of juvenile rheumatoid arthritis there seems to be no good reason to separate Still's disease from other forms.

In young children other manifestations of Still's disease frequently precede the development of noticeable arthritis and sometimes deforming arthritis may not be manifest at all before a spontaneous remission occurs. Therefore early recognition of signs makes possible diagnosis for prognostic purposes and better direction of management.

Clinical manifestations

In the small child illustrated in figure 13 the presumptive diagnosis may be made by noticing the characteristic posture and the facies he presents. He desires to be left alone and remains very still holding his hands and grimacing in a worried fashion. In bed he shows a marked lack of spontaneous movement usually lying on his back with all extremities flexed. These are the signs of arthralgia and myalgia in the small child.

A spiking temperature curve in a patient who acts considerably less ill than would be suspected from the degree of fever suggests the diagnosis of juvenile rheumatoid arthritis; however progression of the disease may lead to severe toxicity and emaciation requiring intensive supportive management, (figure 14). In the older child the primary manifestation is likely to be arthritis of multiple joints with swelling contracture and deformity as in the adult.

Rarely seen in any other disease and therefore a main diagnostic criterion is the spindling of the fingers. This may be recognized early in the disease even in an infant (figure 15).

Figure 13 Worried facial appearance and characteristic posture juvenile rheumatoid arthritis.



Of importance in the diagnosis is the occurrence of a rash that appears to be as diagnostic for juvenile rheumatoid arthritis as the rash of measles is for that disease. The primary lesions are small discrete salmon pink macules with pale centers. Coalescence of the macules frequently makes the rash appear blotchy. This rash is evanescent usually appearing suddenly with fever and disappearing rapidly.

Table 6 shows the frequency of the various clinical manifestations of juvenile rheumatoid arthritis in 75 patients between 6 months and 15 years of age.

Confusion with rheumatic fever often arose when signs of carditis were elicited. Systolic murmurs of grade II intensity were heard not infrequently during the toxic phase of the disease. Tachycardia and non specific electrocardiographic changes consisting mostly of inversion of T2 and T3 and slight prolongation of the PR and QT intervals were seen. No definite heart block or evidence of residual carditis has been seen.

Laboratory findings

Laboratory findings were non specific for juvenile rheumatoid arthritis. The sedimentation rate was elevated, elevation of the globulin caused reversal of the A/G ratio, marked leukocytosis frequently



Figure 14 Cachectic appearance in Still's disease

Figure 15 Spindling of fingers recognizable early in juvenile rheumatoid arthritis detectable even in the pudgy hands of young children



occurred and anemia often was severe. The most common roentgenographic joint abnormality was periarticular osteoporosis. Mild albuminuria occasionally spurred the hunt for indolent urinary tract infection. Bacteriologic, serologic and kin tests helped to rule out arthritis of infectious origin. Except for increased plasma globulin, studies of liver function failed to disclose abnormalities. The concentration of mucoproteins in the serum ranged widely with the mean

Table 6

Clinical manifestations in juvenile rheumatoid arthritis

Symptom	Frequency
Arthralgia	91%
Arthritis of multiple joints	90%
Fever	91%
Myalgia	65%
Swelling of finger	51%
Lymphadenopathy	51%
Skin rash	48%
Hepatoplenomegaly	40%

Table 7

Features important in the differential diagnosis of juvenile rheumatoid arthritis and rheumatic fever

Manifestation	Juvenile Rheumatoid Arthritis	Rheumatic Fever
Arthritis	Indolent Seldom migratory Tend to involve small joints Deforming	Frequent Regularly migratory Tend to involve large joints Never deforming
Fever	Septic temperature curve common	Septic temperature curve typically rare
Splenomegaly and generalized adenopathy	Common	Rare
Rash	Frequent, blotchy erythematous with white streaks	Erythema marginatum Erythema multiforme Erythema infectiosum
Response to salicylates	Poor	Excellent
Valvular heart disease	No	Common
Irregularities of heart rate by stethoscope	Unusual	Regular

value more than three times normal. Titers of antistreptolysin O in the blood were helpful in differentiating juvenile rheumatoid arthritis from rheumatic fever. Of 39 patients only 11 had titers greater than 250 Todd units. The three major L.E. tests were invariably negative (186 tests on 58 patients).



Figure 16 Dramatic response to steroid therapy of patient in figure 14. Note Cushinoid side effects.

Clinical findings in relation to age

Of our patients manifesting lymphadenopathy, hepatosplenomegaly, spiking fever and rash, 86% were less than six years of age. On the other hand, 87% of patients with spiking fever, typical rash and arthritis but without lymphatic hypertrophy had the onset of their manifestations after the age of six years. Patients who had primarily arthritis of the more indolent nature were usually more than six years of age. The marked hypertrophy of the lymphatic tissue in these children has the same age distribution as that seen in chronic diseases known to be caused by infectious agents, and this is interpreted as indirect evidence supporting the possibility that juvenile rheumatoid arthritis has an infectious etiology.

Since etiology and pathogenesis are obscure no rationale for therapy exists. Treatment is aimed at the prevention of joint disability. This should be gentle especially when given with hormone therapy. Rest in bed does not seem to modify the course of the disease and may actually be harmful in contributing to the production of contractures. Salicylates appear to be useful in relieving pain and stiffness. Hormones appear to suppress the disease but do not induce lasting remission. Dramatic results are often experienced with hormone therapy as seen in figure 16. The suppression of the disease produced by adrenal corticoid therapy appears to be related to the levels of 17 hydroxycorticosteroids in the blood (figure 17). However since the disease is characterized by long period of activity complications of hormone therapy are much more troublesome than in rheumatic fever. Treatment therefore is directed primarily toward relieving joint symptoms to facilitate physiotherapy. In cases of inaction hormone therapy is helpful because of its stimulating effect upon the appetite.

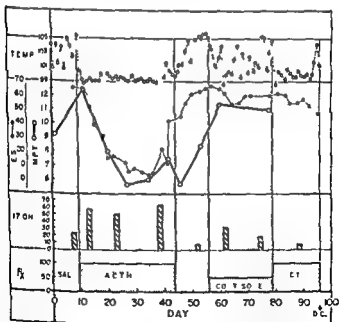


Figure 17 Relationship of plasma 17 hydroxycorticosteroid levels to the suppressive effect of adrenal corticoid hormone therapy on erythrocyte sedimentation rate during process.

Because of apparent hypersusceptibility to infection early specific antibiotic therapy for intercurrent infection is indicated. No deaths resulted from superimposed infection and amyloidosis was not seen in the group studied. With individualized management the prognosis for life in juvenile rheumatoid arthritis is excellent and many patients recover without severe sequelae. However, in some the disease is characterized by repeated attacks and progressive joint disability.

Systemic Lupus Erythematosus

Studies of splenic aspirates

DR MALCOLM M. HARGRAVES. For our hematologic studies we have used smears of material obtained by splenic puncture. The preparations are made on chemically clean slides from the material in the needle itself. Cells developing in the ground substance of the spleen can be rolled out on the slide and are not as disrupted as those from aspirates of bone marrow. Free nuclei may be seen as well as small hyaline bodies that are undoubtedly part of the serum proteins being fragmented off from the ground substance. Compressed erythrocytes can be seen squeezing their way through the sponge like mass of ground substance making up the splenic pulp. The open circulation is apparent.

Figure 18 presents photomicrographs from a case of systemic lupus erythematosus. This and certain other mesenchymal diseases are pathologically so similar to Hodgkin's disease, lymphomas and leukemias that I believe these too should be considered mesenchymal diseases.

Mesenchymal diseases as diseases of environmental origin

I also believe that all of the mesenchymal diseases are primarily environmental in origin. An individual idiosyncrasy appears to be fundamental to the disease process and there is often a long latent period between exposure and appearance of the disease. The precipitating factor may be infection or some similar aggravating incident occurring after the patient has withdrawn from contact with the intoxicating agent.

Chronic poisoning with benzol affords a good example of disease caused by environmental factors. Benzol is capable of producing

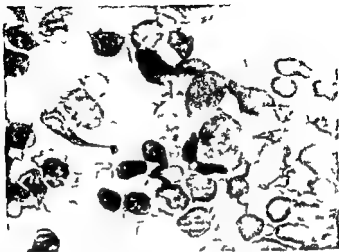


Fig. 11.11. Micrographs of a peripheral blood smear from a patient with systemic lupus erythematosus. Compressed erythrocytes may be seen passing through the interstices. Under reduced light (lower) the heterogeneity of the ground but can be detected in the hyaline bodies so prominent in many of the lymphocytes. A leukocyte may be seen.

aplasia of the marrow hyperplasia of the marrow acute leukemia, chronic myelitis pleromegaly and according to Mallory, other conditions difficult to distinguish from neoplasia Mallory in fact, stated 20 years ago that biologically toxic agents can be roughly classified into two categories—substances that, in adequate dosage will produce their effect on all exposed individuals and substances that, in minute doses will produce in a few individuals devastating effects that cannot be duplicated in the majority of subjects even after administration of large doses of the agent

In leukemia lymphoma systemic lupus erythematosus and the other mesenchymal diseases, the history if carefully elicited is often significant in this connection Classic cases of systemic lupus erythematosus may occur in patients receiving hydralazine hydrochloride (Apresoline®) and may subside when the drug is no longer given Similarly I have seen a number of women with systemic lupus erythematosus who gave histories compatible with sensitivity to permanent wave solutions An epidemic of aplastic anemia once was caused by use of a certain hair preparation

It is intriguing that in the disease there appears to be a synergistic effect of various agents—the association of x ray for example with infection or with administration of benzol compounds

Discussion

DR R A GOOD We have been interested in studying the reactivity of the individual to solvent materials of the sort that were mentioned You can readily sensitize the skin of the rabbit or human to benzol compounds The reaction produced can be classified with the delayed type of sensitivity In other words the π compounds produce reactivity in the human and the animal very much like that of the tuberculin reaction We have recently been able to transfer this sensitivity using leukocytes of the peripheral blood and thus produced generalized allergic reactions

DR HARGRAVES I would like to mention that most paint and varnish removers contain considerable amounts of benzene Hundreds of do it yourself people are being exposed daily in their own workshops I believe the hazard of such exposure is magnified by the increasing likelihood in our medical centers of exposure of the subject to x ray

Dermatomyositis and Scleroderma in Childhood

DR RALPH J WEDGWOOD In reviewing the literature I have come to the conclusion that generalized scleroderma is one of the most rare of all the collagen diseases in childhood more rare than systemic lupus erythematosus, and probably at least as rare as polyarteritis nodosa

Dermatomyositis on the other hand is a relatively common disease More than 100 childhood cases have been described About 60% of the children with this disease survive Since their recovery is limited only by the degree of residual deformity early and correct diagnosis allowing early and adequate therapy should decrease the residual morbidity

Diagnosis of dermatomyositis

Reliance on biopsy for pathologic diagnosis of dermatomyositis in my experience unsatisfactory Not always do we see the classic changes in the muscle bundles with vacuolization centralization of nuclei degeneration infiltration between bundles and perivascular changes Early in the disease pathologic findings are usually only confirmatory not diagnostic

I should like to describe a few symptoms and signs in dermatomyositis which are so peculiar to that disease that they might constitute major criteria a for example a diastolic murmur in the aortic or mitral area can constitute a criterion for the diagnosis of rheumatic fever The frequency of the most important signs and symptoms of dermatomyositis are listed in figure 19 Four are of particular value in diagnosis weakness, infiltration of the face facial lesion and pain and tenderness

The onset of the disease is extremely insidious and often the first sign is weakness or easy fatigability The weakness in children is usually first apparent in the legs, although involvement of the shoulder girdle often exists earlier and may be more severe Eventually the weakness and fatigability become so obvious that advice is sought Involvement of the muscles of respiration and of the palate are of particular importance Prior to the days of therapy with steroids almost all the fatalities occurred because of such palatorespiratory involvement

Induration of the tissue is more difficult to describe. The involved muscles and subcutaneous tissues in dermatomyositis have a particular feeling on palpation variously described as thickened leathery indurated brawny rubbery or inelastic. With satisfactory relaxation in the patient the muscles of the shoulder girdle particularly the superior portions of the trapezius in the neck are likely to demonstrate this phenomenon.

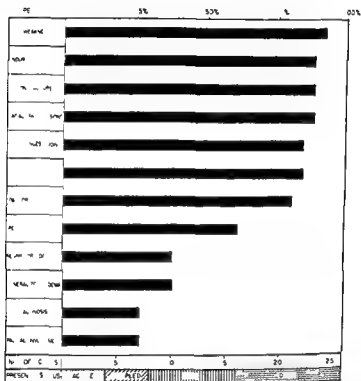


Figure 19 The incidence of various signs and symptoms in children with dermatomyositis. Reprinted from *Pediatrics* 12:418 (October) 1953.

The third important finding is the butterfly rash of the face which usually is no more than a slight erythema and induration with occasional scaling of the malar areas and the bridge of the nose. It is rarely as severe as may be seen in systemic lupus erythematosus. The butterfly distribution of the rash is less significant as a diagnostic sign than the violaceous heliotrope or faintly erythematous discoloration that becomes apparent on the upper lids of the eyes. This

sion is usually present in dermatomyositis and occurs rarely if ever in other diseases with which it might be confused. It may or may not be associated with periorbital edema.

The child's pain in dermatomyositis is frequently localized by the parents to the joints. The children themselves usually localize the pain in the muscles. It occurs on movement, occasionally at rest and occurs at the tendinous area of the muscle rather than in the joint proper. This finding allows differentiation from the arthralgia of the other mesenchymal diseases. Tenderness may also be demonstrable in the muscle bundle itself. The same findings and the almost imperceptible onset of the disease differentiate it from viral myalgia or trichinois.

Manifestations in scleroderma

In scleroderma, skin binding might be considered one major diagnostic sign. Others might be the stiffening of the finger, visceral changes, particularly of the esophagus, and cardiac findings which in adult are apparently more frequent in scleroderma than in dermatomyositis. No data on the frequency of occurrence of these changes are available as far as I can find for the pediatric age group.

Therapy in dermatomyositis

Functional recovery from dermatomyositis depends on prevention of contractures and correction of the deformities that cannot be prevented. To this end there is no substitute for vigorous physiotherapy planned carefully, supervised well and made acceptable to parents and patient. Physiotherapy should be instituted in spite of active disease. Orthopedic assistance should be sought early to prevent contractures. Early amputation is probably the optimal form of therapy.

Steroids have been used and with certain reservation have apparently aided therapy by providing symptomatic relief. In our experience they have not altered the mortality. In some of these patients the symptomatic relief is quite striking, allowing early physiotherapy and early amputation. There is also a return of muscular function which may be due to a decrease in pain but probably also involves actual increase in muscular power.

Prior to the availability of steroids, children died mainly because of palatorespiratory involvement. Of the children to whom we have given steroid, six have died only on the basis of palatorespiratory involvement alone. Two died of respiratory involvement and another factor. This factor is probably the major cause of death in three other patients as well as a contributory cause in these two was a change in

the gastrointestinal tract. Five of the six children suddenly developed gastrointestinal crises with perforation of unsuspected ulcer, paralytic ileus and gastrointestinal hemorrhage.

Gastrointestinal lesions in dermatomyositis described prior to the availability of steroids do not seem to have been as severe as those seen in children who die while receiving steroid therapy. These changes are probably an accentuation of the vascular changes seen elsewhere in the body in this disease and are probably intrinsic to the disease itself.

Nephrosis and Nephritis in Childhood

DR CAROLYN F. PIEL. Nephritis and nephrosis although not generalized, do involve vascular mesenchymal tissue and hence warrant discussion with the mesenchymal diseases.

General considerations

Acute glomerulonephritis and the nephrotic syndrome are generally recognized as distinct clinical entities in the pediatric age group. The frequent occurrence of the nephrotic syndrome in patients with known chronic glomerulonephritis and the finding of histologic changes indistinguishable from those of chronic nephritis in the glomeruli of patients with nephrosis have led to the close association of the two illnesses in the minds of pathologists and investigators.

The two diseases are differentiated by age of onset, the inciting role of certain strains of beta hemolytic streptococcus in acute glomerulonephritis and the prominence in that disease of hematuria, azotemia and hypertension in contrast to the hypoproteinemia, hyperlipemia, anasarca and greater degree of proteinuria in nephrosis. Renal clearances of inulin and paraaminohippurate are often diminished in acute glomerulonephritis and tend to be normal or supernormal in nephrosis. In addition, the prognosis is much more favorable in acute glomerulonephritis than in nephrosis.

There are indications that acute glomerulonephritis is a disease of allergic origin. These include a known specific inciting agent, the beta hemolytic streptococcus; the latent period of 10 to 14 days between the acute streptococcal infection and the onset of nephritis; the excessive increase in titer of antistreptolysin O and the decreased titer of complement in the serum. In addition, glomerulonephritis comparable to the human disease can be produced in animals by various immunoallergic procedures.

Nothing can be said about the period of latency for development of nephrosis since the inciting agent is not known. Most observers have found that the titer of anti streptolysin O is low in patients with nephrosis who are edematous. The suggestion that this low titer results from the loss of antistreptolysin O in the urine has not been confirmed.

The concentration of complement in the serum is reduced in both diseases a finding interpreted by some as evidence that nephrosis is a disease of antigen antibody reaction. However determinations of the concentration of complement are not reliable in the presence of

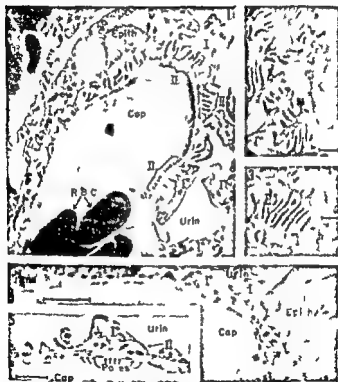


Figure 9. Low power electron micrograph of glomerular capillary lumen (Cap) and urinary space (Urin) showing epithelial foot processes (Epith) and primary (I) and secondary (II) lamellae. The foot processes are digitate as seen in 3 and are separated distally in the basement membrane of the capillary loop by endothelial cells. Section 4 demonstrates the endothelial cell (End.) also separated from the basement membrane by the basement lamellae, and 5 the system of pores that traverse the endothelial sheet. R print of Am. J. Med. Res. 11: 12, 1955.



Figure 21 Electron photomicrographs of the kidneys of animals fed silver nitrate in their drinking water for many months prior to experimentation (Upper) Section from a control animal not treated with nephrotoxic serum. The silver can be seen as black dots within the basement membrane. A few tiny dots can be seen in Bowman's capsule and occasionally some can be seen in the basement membrane of the tubules. (Lower) Section from an animal that had received nephrotoxic serum three days previously. A prominent increase in epithelial substance may be noted as well as some thickening of the basement membrane. The foot processes have almost lost their identity.

lipemia and the possibility of loss of complement in the urine makes the evaluation of any change in concentration of complement difficult. Two other findings are perhaps more suggestive of an allergic basis for nephrosis. Localization of gamma globulin in the basement membrane of the glomeruli of patients with nephrosis has been demonstrated and an experimental model of nephrosis can be produced by immunoallergic techniques.

Experimental renal disease in animals

Two types of nephrotoxic serum are used to produce experimental disease. Serum prepared by injecting emulsion of kidney from another species into ducks or hen and serum prepared in rabbits. It has been repeatedly demonstrated that the specific antigen lodges in the basement membrane of the glomerulus. It is thought to be protein bound with polysaccharide or lipid. Nephrotoxic serum prepared in rabbit, injected in adequate dose, produces disease immediately. A depression of complement also occurs but is apparently non specific since it occurs also in nephrectomized animals.

We are currently engaged in studying the glomerular changes in renal diseases in humans and in experimental animals (studied by light and electron microscopy). Figure 20 presents a micrograph of the glomerulus as seen with the electron microscope and figure 21 presents electron photomicrographs of the kidneys of animals fed silver nitrate in their drinking water for many months. The changes seen three days after administration of nephrotoxic serum may be compared with the histologic appearance in the control animal. The cement substance is lost soon after administration of nephrotoxic serum but in the animal that have received silver nitrate cement substance cannot be visualized even prior to treatment with nephrotoxic serum. Three weeks after administration of nephrotoxic serum the foot processes of the epithelial cells are somewhat restored cement substance can be seen and yet there persists a thickening of the basement membrane.

Electron microscopic examination of renal biopsies of children with nephrosis indicate that the alteration in the epithelial layer loss of identity of foot processes, absence of cement substance and thickening of the basement membrane parallel closely the changes seen in the renal disease produced in rats by administration of nephrotoxic rabbit serum. Some of these changes disappear with clinical remission induced by steroid therapy.

Renal Biopsy in the Study of Chronic Renal Disease In Children

DR ROBERT L. VERNIER We have examined by light microcopy specimens of kidney tissue obtained at laparotomy in 12 patients and by percutaneous needle aspiration in more than 90 other children with renal disease. Electron microscopic study of approximately one half of these specimens has been completed in cooperation with Farquhar of the Department of Pathology, University of California, San Francisco. From our experience it appears that 10 or more glomeruli and associated tubules are adequate for diagnosis in the group of diffuse renal diseases we have studied.

The specimen is obtained under local anesthesia with the patient in the prone position. The Franklin modification of the Vim Silverman needle is used. The only complication we have encountered is the formation of a small perirenal hematoma in a three-year old child with the nephrotic syndrome. There are no recognizable sequelae of this complication. Although most children have microscopic hematuria for a short interval after this procedure, only two instances of brief gross hematuria have been observed. No significant alterations in the renal clearance of inulin have been measured when this study was performed before and after renal puncture. The children tell me the procedure hurts less than apiration of the bone marrow.

Findings by light microscopy

In our recent study of 25 cases of anaphylactoid purpura, Addison counts indicated that 70% of the children had renal involvement. We have studied renal specimens from seven of these children and have found that the renal lesion is characterized by proliferation of endothelial cell and focal fibrinoid occlusion of the glomerular capillaries. There is tremendous variation in pathologic findings from patient to patient depending on the duration and severity of the disease at the time of the biopsy. The morphology of the renal lesion in some patients resembles that seen in systemic lupus erythematosus.

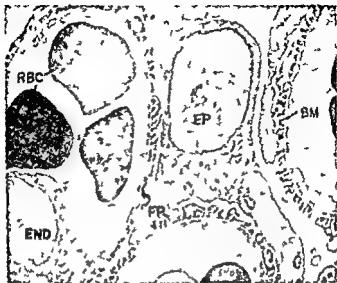


Figure 2. Electron micrographs of sections of glomerular loops in biopsies. (Upper) Normal glomerular loop showing the epithelial cell (EP) with its foot processes (FP), the basement membrane (BM) and an endothelial cell with its fenestrated endothelium (END). (Lower) Loop as seen in so-called pure nephrosis, demonstrating the striking loss of epithelial foot processes. The basement membrane is essentially normal but the endothelial cytoplasm appears swollen.

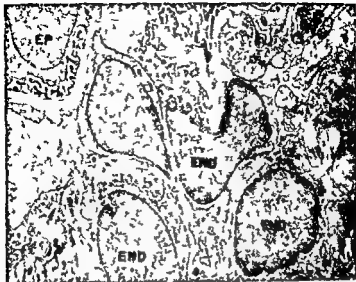


Figure 23 Electron micrographs of section of renal biopsy in children (Upper) Lesions seen in acute glomerulonephritis. The most characteristic findings are a marked endothelial proliferation and a beginning accumulation of material which resembles basement membrane in density. The epithelial foot processes are essentially normal. (Lower) Lesions seen in chronic glomerulonephritis. The lumina of many of the glomerular loops have been obliterated by endothelial proliferation and accumulation of basement membrane like material.

In systemic lupus erythematosus the renal involvement is often severe and is frequently the cause of death. The classic pathologic lesion is said to be the wire loop alteration of the arterioles. In our studies by light microscopy the renal lesion of systemic lupus erythematosus was often pathologically indistinguishable from the lesion of anaphylactoid purpura or glomerulonephritis in its various forms. In general there was considerable variation from minimal endothelial proliferation and focal glomerular occlusion by fibrinoid to severe hyalinization. No characteristic wire loops were seen. Some evidence of reversal of proliferative changes after treatment with cortisone was noted in one case.

Glomerulonephritis

We studied a 12 year old boy 10 days after the onset of sore throat fever and lymphadenopathy. Hypertension and hematuria were present and the beta hemolytic streptococcus type 12 was cultured from the throat. A clinical diagnosis of acute glomerulonephritis was made. There was no history of previous renal disease. On biopsy two types of lesions were found one acute compatible with the classic description of glomerulonephritis. There were large numbers of polymorphonuclear leukocytes in many of the glomeruli, triking proliferation of endothelial cells and glomerular ischemia. However chronic changes were also present consisting of glomerular hyalinization, considerable tubular atrophy and interstitial infiltration. The chronic changes appeared to antedate the clinical history of onset by many weeks. One month later the proliferative changes had been ameliorated but the chronic changes persisted. The patient's course was that of chronic nephritis.

The pathologic findings indicated that this boy had unrecognized renal disease of insidious onset, and suffered an acute exacerbation through infection by this strain of streptococcus. Had the patient not been studied by biopsy the sequence of events in this case would have appeared to support the view that acute glomerulonephritis can progress to chronic glomerulonephritis. Perhaps some of the discrepancies in the literature regarding the relationship between these diseases will eventually be resolved by studies of this type.

I think that chronic glomerulonephritis is a pathologic end state the etiology of which we do not know. It may have several. It is probably no more specific a diagnosis than cirrhosis of the liver and indicates little of the nature of the inciting agent.

Nephrotic syndrome

From our experience with the nephrotic syndrome we think that the renal lesions are related to both the severity and the duration

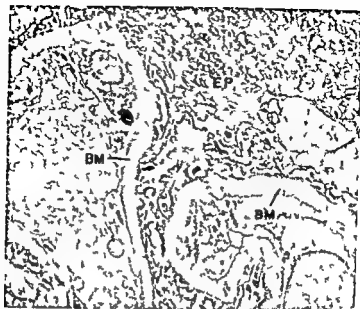


Figure 24 Electron micrograph of a section of renal biopsy in a child. Lesions seen in systemic lupus erythematosus. The most striking abnormality present early in the disease is a generalized thickening of the basement membrane proper. Here the basement membrane is four to six times normal thickness.

of the disease. Early in the course of the disease the kidney appears normal by light microscopy. If the disease has persisted for many months there is likely to be considerable scarring with many hyalinized nonfunctioning glomeruli despite the absence of clinical and laboratory evidence of nephritis.

At times the renal lesions of children with nephrosis with subacute glomerulonephritis and with systemic lupus erythematosus may be strikingly similar by light microscopy. We have utilized the electron microscope in the study of the early ultramicroscopic morphologic alterations that occur in the various diffuse renal diseases.

Findings by electron microscopy

With the electron microscope three basic components of the glomerular capillary—epithelium, basement membrane, and endothelium—are readily seen. The three distinct membrane layers are shown to be differentially involved in nephrosis, glomerulonephritis, and the nephritis of systemic lupus erythematosus.

The ultramicroscopic pathology of the nephrotic syndrome is characterized by distortion and smudging of the foot processes of the epithelial cell. The basement membrane proper and endothelium remain essentially unaltered early in the disease. This marked alteration of the epithelial cell is present regardless of the clinical state of the disease or the severity of change as seen by light microscopy.

In glomerulonephritis, the endothelial cells undergo marked proliferation and variable quantities of a material which resembles basement membrane appear in intimate relationship to the cytoplasm of the endothelial cells. The epithelial cells are normal except in very severely involved glomeruli.

Systemic lupus erythematosus is associated with a renal lesion characterized by thickening of the basement membrane proper. The endothelium and epithelium are relatively normal and become significantly distorted only late in the process.

Discussion

DR. EHRICH: In animals it is possible to study the pathogenesis of these various lesions step by step under controlled conditions. On the basis of such studies I should like to offer an alternative interpretation of the changes demonstrated by Vernier. I believe that many of the lesions shown to us were complications of the underlying disease consisting of thrombosis of glomerular loops and eventual reorganization of thrombotic material. In many cases the thrombosis had led to leakage of fibrinoid exudate into the capillary space which became organized with the formation of crescents. These complications are seen in all glomerular diseases with the exception of amyloidosis. We see them in systemic lupus erythematosus, in glomerulonephritis and in nephrosis.

DR. VERNIER: I fully agree that the thrombotic manifestation seen in light microscopy may be a complication of the basic disease process. I do not believe I can separate the complications from the disease. However the three basic components of the glomerular capillary as seen by electron microscopy are differentially involved in the three diseases. Although the changes may be somewhat sparse or varied from one glomerulus to the next changes in the endothelium, epithelium and/or basement membrane make it possible to distinguish the various pathologic processes.

All our current conclusions should certainly be considered tentative. I think these changes indicate that the renal expression of a disease may depend on the particular component of tissue involved early in the pathogenesis of the disease. The injury to the

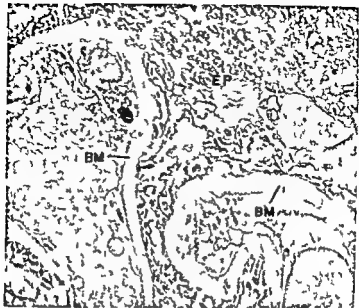


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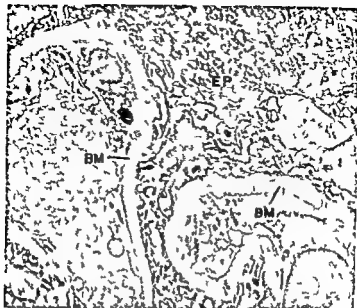


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epithelium per se results in massive proteinuria and development of the nephrotic syndrome injury to endothelial cell results in gradual development of glomerulosclerosis, hypertension and cetera

DR R A GOOD Experimental models are not always adequate for the purpose. Sometimes relying on experiments of nature provides an even more incisive approach than does experimentation with animals. We have had an unique opportunity to study an interesting experiment of nature in which four children in one family developed the nephrotic syndrome early in life. From the clinical point of view the diseases that these children presented at the time we studied them were respectively transient pure nephrosis, persistent pure nephrosis, mixed nephrosis and nephritis and chronic glomerulonephritis. We can assume although there might be some doubt about the validity of the assumption that these children all suffered from the same basic etiologic process.

Pathologically using the light microscope the entire clinical spectrum of renal disease was revealed. The two children with pure nephrosis had no demonstrable renal lesion. The child with mixed nephrosis and nephritis showed pathologic evidence of proliferative glomerulonephritis. The other child pathologically as well as clinically had typical chronic glomerulonephritis.

All of these kidneys when studied by the electron microscope showed the same basic lesion namely the smudging of the foot processes along the basement membrane proper that Vernier has illustrated. We have been forced from this observation to conclude that the several forms of the nephrotic syndrome diagnosed by clinical criteria and pathologic changes seen by light microscopy are in reality manifestations of a single basic disease process an early concomitant of which is the disturbance in morphology of the epithelioid cell podocytes that has been revealed by electron microscopy.

Anaphylactoid Purpura in Children

DR THEODORE C PANOS My purpose is to review the syndrome of anaphylactoid purpura to present some pertinent case material and to discuss the similarities between this disease and other mesenchymal diseases.

Designations and major manifestations

The disease is designated by a variety of names. Henoch Schonlein syndrome is preferred by Gairdner. allergic purpura is preferred

ly Wintrobe and acute vascular purpura by Dameshek. The most popular designation seems to be anaphylactoid purpura. Vascular anaphylactoid purpura and primary purpura are other synonyms.

The disease is a polymorphic or protean recurrent disease due to hypersensitivity and resulting in a generalized disturbance of small vessels. It is characterized by the following major manifestations occurring in any combination or sequence: a macular papular or petechial or purpuric rash, gastrointestinal signs and symptoms including colic, vomiting, melena, diarrhea and intussusception, and involvement of joints manifested by pain and swelling. It has frequently been stated that the presence of any two major manifestations constitutes a basis for positive diagnosis. Fever when present is usually low grade. Renal involvement is often conspicuous and varies from microscopic hematuria to gross hematuria with hypertension, azotemia and uremia.

In order to delineate more satisfactorily the relationships among the various manifestations, we undertook an analysis of 572 cases occurring in children less than 15 years of age and reported in the English language during the past 20 years. Well over 90% of these cases and two thirds of the publications have appeared since 1951. The incidence of the disease has definitely increased during the past year.

Frequency of various symptoms and signs

The sex distribution is 3 to 2 with predominance in males. The age at onset ranges from 8 months to 15 years, the highest incidence occurring between 3 and 12 years, with the median and mean at about 5 years. The duration of the acute phase is from 6 to 180 days for the great majority of cases, with a median of about 21 days. At least one recurrence can be expected in about one third of the cases. One half of the recurrences were single and most took place within 2 to 12 months of the first attack. Only rarely did the interval exceed six months, although occasionally as much as two years elapsed between episodes. Infections are very often cited as precipitating factors in relapse. The mortality is about 2% mainly from renal failure.

Individual case histories were sufficiently detailed in 9 instances to allow the tabulation of frequency of occurrence of certain signs, symptoms, and antecedent circumstances. Antecedent infection occurred in 33 cases or 37%. Thirty-two of these infections were of the upper respiratory tract, and *Streptococcus hemolyticus* was cultured from nearly half of the patients. The most commonly stated interval between infection and onset of disease was 7 to 14 days. A nine year

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Individual case histories were sufficiently detailed in 89 instances to allow the tabulation of frequency of occurrence of certain signs, symptoms and antecedent circumstances. Antecedent infection occurred in 33 cases or 37%. Thirty-two of these infections were of the upper respiratory tract and beta hemolytic streptococcus was cultured from nearly half of the patients. The most commonly stated interval between infection and onset of disease was 7 to 14 days. A nine year

study by Lewis of 139 cases of anaphylactoid purpura 290 of glomerulonephritis and 297 of rheumatic fever elicited comparable incidence of antecedent upper respiratory infection—50% 46% and 64% respectively. He was able to culture hemolytic streptococcus from one fourth of the patients in each group.

Pat history of allergy was elicited in 8% of the 89 cases, family history of allergy in 13% an incidence not significantly greater than in the general population. Foods were specifically implicated as an inciting factor in 11 cases. The most common items mentioned were chocolate egg albumin tomatoes pork and fish. Insect bites were implicated in three cases. Drugs were specifically mentioned only in two cases in each case penicillin.

The frequency of signs and symptoms in these cases listed according to systems is given in figure 25. Forty eight were described in sufficient detail to allow a tracing of the sequence of appearance of signs and symptoms. In decreasing order the most common initial complaints were colic purpura swelling of the joints vomiting pain in the joints and petechiae. Fever was not a prominent early finding.

Renal involvement deserves special comment because of its frequency and because it is the most common cause of death. Hematuria appeared within the first week in two thirds of the cases. Azotemia and/or hypertension occurred in 40 to 50% of the patients with gross hematuria, a finding present in one fourth of all patients with renal involvement. Although the vast majority of patients recovered promptly there was a tendency in some for microscopic hematuria to persist from several months to several years perhaps indicating latent or chronic nephritis.

It was possible to find reports of treatment with either adrenocorticotropin or cortisone in only 42 cases. In 14 the results were described as excellent in 10 as fair and in 8 as poor. Exacerbation after withdrawal of steroid therapy occurred in 17 patients and 10 required retreatment. In patients with renal involvement the steroids were conspicuously ineffective.

Relationship between anaphylactoid purpura and polyarteritis nodosa

Ever since Osler in 1914 drew the analogy between serum sickness and anaphylactoid purpura and predicted that before long the anaphylactic key would unlock the mystery of these cases the tremendous interest has been generated in the relationship of the anaphylactoid purpura syndrome to mesenchymal diseases. Similarities between anaphylactoid purpura nephritis rheumatic fever and polyarteritis nodosa were recognized early as was the fact that differentiation among these entities is occasionally virtually impossible at certain

stages of their development. Gairdner in 1948 grouped anaphylactoid purpura with rheumatic fever, nephritis and polyarteritis nodosa to form a family of diseases that he considered to be linked together by the tendency for one to coexist with another and by characteristics common to the group. He also contended that anaphylactoid purpura and polyarteritis nodosa are essentially the same disease process, capillaries being involved in the former, medium sized arteries in the latter, with arteriolar involvement common to both.

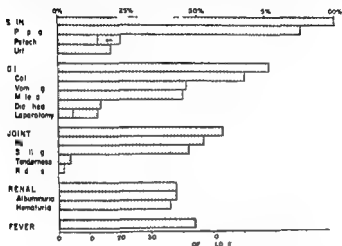


Figure 25. The frequency of various signs and symptoms in anaphylactoid purpura children.

Actually there is a surprising paucity of pathologic material on anaphylactoid purpura and most of the evidence linking this disease with polyarteritis nodosa is circumstantial. Even histologic studies, although strongly suggestive are not conclusive.

However, the following similarities do provide evidence of an interrelationship: both are generalized vascular disorders with aseptic inflammation of the vessels of skin, gastrointestinal tract, joints and kidney and this vascular involvement is sufficient to explain the clinical picture. Necrotizing arteriolitis occurs in severe forms of anaphylactoid purpura. Both are related to antecedent infection in a manner suggesting that hypersensitivity plays a role. Also in a child, the progression of anaphylactoid purpura into classic polyarteritis nodosa has been reported. Polyarteritis nodosa can readily be produced by injections of horse serum and at least the major manifestation of anaphylactoid purpura can be reproduced in the same manner.

Arguments can be raised against the likelihood that anaphylactoid purpura and polyarteritis nodosa are closely related diseases. The lesions are not identical. In anaphylactoid purpura there is an infiltration about the capillaries rarely about arterioles and then only in the very severe and/or fatal cases. Polyarteritis nodosa begins with and is characterized by segmental necrosis of the medium sized arteries and there is a paucity of lesions in capillaries and veins. Nodosa lesions have not been reported in anaphylactoid purpura. The rarity of cardiac lesions in this disease contrasts with the incidence of 84% in polyarteritis nodosa. Renal involvement is much more common in polyarteritis (75%) than in anaphylactoid purpura (35-40%). The reversibility of the lesion of anaphylactoid purpura is in contrast to usual irreversibility in polyarteritis nodosa. Polyarteritis is ordinarily fatal whereas anaphylactoid purpura is rarely fatal. However it seems to me there is no doubt that anaphylactoid purpura belongs to the family of mesenchymal diseases.

Hormone Therapy in Rheumatic Fever

DR ALAN A. DONE While there is general agreement that administration of hormonal agents and salicylates produces more or less prompt subsidence of the acute symptoms of rheumatic fever the opinion that none of them alters the frequency of cardiac sequelae has been advanced repeatedly. These conclusions have been based on data collected during studies with a limited variety of therapeutic regimens very few of which were individualized in any way. There would seem to be little reason to believe that adrenocorticotropin and cortisone in contrast to other medications can be used without regard for response of the condition under treatment.

Individualization of hormonal therapy

In our studies individualization of therapy was accomplished by 1) adjusting the size of the dose to the size of the patient that is 1 I.U. of adrenocorticotropin or 3 mg of cortisone per pound of body weight per day as the minimum initial dose 2) adjusting the size of the dose to the severity of the illness severely ill patients receiving a larger initial daily dose and 3) adjusting the duration of therapy according to the response of the patient. Therapy with the full initial dose is continued not for a fixed period of time but until there is no longer any clinical and laboratory evidence of activity of

the disease. In patients treated early during their attacks of rheumatic fever this initial maximal dosage is usually given for two to four weeks.

Because the clinical signs of disease disappear rapidly under the influence of hormones it is difficult to arrive at a satisfactory definition of the proper duration of therapy with the initial maximal dose. The erythrocyte sedimentation rate and concentration of C-reactive protein in the serum are not satisfactory criteria since these values become normal too soon after the initiation of therapy. Discontinuation of treatment at that point is likely to result in clinical relapse.

There is little hazard of clinical relapse if hormonal therapy is continued in maximal dosage until the concentration of mucoprotein in the serum has decreased to less than 6 mg/100 ml and if tapering of the dosage is done gradually and cautiously. In our clinic therapy with the full initial dose is continued until the following criteria have been met: all clinical evidence of activity has disappeared, the erythrocyte sedimentation rate has been normal for at least one week, and the concentration of mucoprotein in the serum has decreased to 6 mg/100 ml. The dosage is then gradually reduced at intervals of two to three days. Before each reduction of dosage the erythrocyte sedimentation rate is determined. If there is any evidence of relapse the dose is not further decreased until this evidence has disappeared.

Effect of hormonal therapy on incidence of residual cardiac damage

The measure of efficacy of any treatment for rheumatic fever must be based principally on the production of a lower incidence of residual cardiac damage. Unfortunately this effect is the most difficult to assess. In patients who have sustained heart damage from previous rheumatic fever or in whom the prior cardiac status is unknown such evaluation is nearly impossible.

A comparison has been made of the incidence of residual cardiac murmurs in 85 patients who were known to have no murmur before the onset of an attack of acute rheumatic fever and who had exhibited signs or symptoms of rheumatic fever for less than three months. Of these 49 were treated either with adrenocorticotropin or cortisone and 36 with salicylates or bed rest alone. All these patients had unequivocal evidence of carditis prior to therapy. Patients who exhibited evidence of recurrence of rheumatic fever were included in the follow-up study only to the time of the last examination prior to the recurrence. The findings in hormone-treated patients are grouped and compared with those treated with salicylates or bed rest alone since no significant difference in incidence of residual cardiac murmurs was found between the groups treated with adrenocorticotropin

or *continue* one or between those treated with salicylates or merely bed rest

Table 6 presents data on the incidence of diastolic murmurs or systolic murmurs of grade 2 or greater intensity at intervals after cessation of therapy. Among the patients not treated with hormones the percentage exhibiting diastolic murmurs or systolic murmurs of grade 2 or greater intensity ranged from 42 to 65 throughout the four years of follow up. In contrast among the patients treated with hormones the incidence declined to 9% within three months after discharge and at four years was 6%. The results of application of a similar regimen of hormonal therapy in cases of recurrent and chronic rheumatic fever have likewise been encouraging. Not only did fewer murmurs persist among the hormone treated patients but these individuals developed new murmurs during the follow up period far less frequently than did patients treated with salicylates or merely bed rest

Table 8

The incidence of diastolic murmurs and of systolic murmurs of grade 2 or greater intensity at intervals after a first attack of acute rheumatic carditis

	Acute Ph	At Dis- charge	3 mo	6 mo	1 yr	2 yr	3 yr	4 yr
Hormone treated								
% with murmur	99	23	9	5	7	6	4	6
Treated with salicylates or bed rest alone								
% with murmur	95	50	58	42	45	56	65	60

Adverse effects of hormonal therapy

Among patients with rheumatic fever whom we have treated with hormones during the past five and one half years the only apparent adverse effects of therapy have been the temporary occurrence of facial changes suggesting Cushing's syndrome, excessive appetite and the appearance or aggravation of acne. In a few instances girls have developed urinary tract infections and a number of patients have developed paronychia. These infections have not seemed inordinately severe and have responded readily to administration of antibiotics or chemotherapeutic agents. Significant hypertension has not developed although we have observed it in younger patients who have received

larger doses of hormones in the treatment of hematologic disorders, and in a number of patients with renal disease. In no patient with rheumatic fever have we found it necessary to discontinue the therapy because of side effects.

These observations suggest that it is possible to treat most patients with rheumatic fever with sufficient doses of adrenocorticotropin or cortisone to achieve maximal benefit without encountering serious complications. This does not imply that serious complications should not be anticipated and it should be borne in mind that similar doses of hormones have resulted in serious complication when used in treatment of diseases other than rheumatic fever. It is our impression that the nature of the patient's illness is an important factor in determining what dosage of hormone will be tolerated.

Among the patients treated with adrenocorticotropin or salicylates there appeared to be no significant reduction in responsiveness of the adrenal gland to administration of adrenocorticotropin after cessation of therapy. Significant depression of adrenocortical response did occur after therapy with cortisone but persisted for less than one week.

Discussion

DR LEONARD M. LINDR (Los Angeles, California): How do you account for the lack of mortality in both the treated and untreated patients?

DR DORF: In the group of patients treated with salicylates, there was one death 14 months after cessation of therapy. The fact that none of the patients had previous cardiac damage is probably responsible for the low mortality rate. As you know, the mortality rate in first attacks of rheumatic fever and in recurrent attacks where previous damage to the heart has not occurred is relatively low.

DR SMITH: What was the method of selection of patients for inclusion in the various groups?

DR DORF: Patients are currently selected at random for inclusion in the various groups. Initially this was impossible because a significant percentage of patients was referred specifically for hormonal therapy. Because most of the patients treated with hormones in the early part of the study were referred, the severity of the illness was probably greater in this group, making the results perhaps more significant.

DR DORFMAN: I am quite convinced from what we have observed that it is erroneous to assume that the patients who received salicylates can be considered an untreated control group. Salicylates

have a rather marked effect on certain manifestations of rheumatic fever although I do not know whether they affect carditis

I agree that treatment with a fixed regimen has real disadvantages. I would however point out that in the published data of Done and Kelley the average amount of drug given was similar to that used in the Cooperative Study

DR DOWE It is true that the majority of patients in the Cooperative Study received the same total dose of hormones as did our patients. I think more important than the total dose however is the dosage level at which these patients were treated relative to the duration of their disease. It is important that the initial dose be sufficient to produce the desired response and that dosage be continued at that level until all evident disease activity has subsided. Tapering the dosage gradually with the guidance of the clinical course and laboratory studies was probably a significant factor. Tapering was stopped at any point where clinical or laboratory evidence of relapse was noted.

DR R. A. GOOD We do not see patients with rheumatic fever as early as Kelley, Done and their coworkers do. I presume this is largely a function of the geographic location and the relationship of the general physician to the medical center. Although we have not studied a large series I think negative observations drawn even from relatively few patients have pertinence. For example we have observed the development of new murmurs and have watched progression of rheumatic fever to fatal termination with overwhelming rheumatic carditis even in patients receiving large doses of adrenocorticotropin or cortisone. We are not at present convinced that the carditis can be terminated or uniformly favorably affected by treatment with adrenal steroids.

I am cognizant of the fact that data are accumulating in the literature supporting the position taken by Done and Kelley and their group which indicate that if these patients are treated early with enough hormone more is accomplished in treatment of carditis than was accomplished in the Cooperative Study or in most of the patients that we have seen at Minnesota.

I think it unusual that Done using the dosages described has not observed serious untoward reactions to the hormones in patients with rheumatic fever. Certainly other investigators have observed such complications (Dorfman et al). We have observed severe infections, psychoses of the most malignant type, convulsions, ulcers and severe damage to blood vessels and connective tissue in subjects with rheumatic fever who were treated with large doses of hormone. The recent observations of Eley in Boston that several children under treatment with steroids in moderately large doses succumbed to infection with chickenpox needs only to be mentioned to warn us that

in patients with rheumatic fever as well as those with other diseases the use of these hormones may be hazardous

DR KELLEY Good implied that the regimen of hormonal therapy that he uses is the same as ours. However in our regimen whenever a patient's condition is becoming worse we increase the dosage of hormone. When a patient is critically ill we administer cortisol intravenously to no limit except improvement of the patient.

Since we started this policy we have never had a death result from rheumatic fever in our hospital. Previously there had been no year without such deaths in the records of our hospital. Ours is an area of high incidence of severe rheumatic fever and this improvement occurred despite the increased number of cases seen since the institution of our present program.

Serious Untoward Effects of Hormone Therapy

DR ROBERT A. GOOD It has become increasingly apparent that adrenocorticotropin and cortisone are extraordinarily potent pharmacologic agents, affecting mammalian physiology in several different ways. It is being realized gradually that their effects are not all beneficial. Many of the biologic actions of adrenocorticotropin and cortisone reproducible in the laboratory might be expected to present extreme hazards applied to human beings. Both adrenocorticotropin and cortisone must be looked upon as two-edged swords capable of doing much harm if wielded indiscriminately.

The overenthusiasm of physicians regarding the usefulness of these compounds is best exemplified by the popular practice of reporting in great detail the beneficial effects of their clinical utilization. Description of consequences that are often life-threatening or even fatal are relegated to the final paragraph or a footnote. Few papers are devoted to the description and discussion of untoward reactions to adrenocorticotropin and cortisone while literally hundreds extol their virtues.

Serious consequences of therapy in 37 of 310 children treated

During the past three years we have used adrenocorticotropin and cortisone extensively in the treatment of 310 children, nearly

have a rather marked effect on certain manifestations of rheumatic fever although I do not know whether they affect carditis

I agree that treatment with a fixed regimen has real disadvantages I would however point out that in the published data of Done and Kelley the average amount of drug given was similar to that used in the Cooperative Study

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Serious consequences of therapy in 35 of 340 children treated

During the past three years we have used adrenocorticotropin and cortisone systematically in the treatment of 340 children nearly

Table 9

Serious complications of administration of adrenocorticotropin and cortisone to children: disturbances of equilibrium of fluids and electrolytes and disturbances of the central nervous system

Patient	Complication	Dose Used Treatment	Hormone Implied	Dose mg/ m/d y	Dose m t (d y)
Disturbances of equilibrium of fluids and electrolytes					
DN 4 yr male	Pulmonary edema associated with generalized retention of fluid	Rheumatoid arthritis	ACTH	150	21
HL 8 yr male	Severe generalized edema, ascites and hypertension	Dermatomyositis	Delta cortisone	75	21
RS 9 yr male	Hypopotassemic shock after diuresis	Nephrotic syndrome	ACTH	130	14
NS 4 yr female	Hypopotassemic shock after diuresis during ACTH treatment	Nephrotic syndrome	ACTH	130	14
TG 2 yr male	Hypopotassemic shock after diuresis during ACTH treatment	Nephrotic syndrome	Delta cortisone	75	14
Disturbances of the central nervous system					
ER 13 yr female	Central retinal artery thrombosis, cavernous sinus thrombosis, blindness in right eye	Rheumatoid arthritis	ACTH	77	14
LH 16 yr female	Paranoid schizophrenia	Plasma cell hepatitis	Cortisone	375	30
SO 13 yr female	Paranoid schizophrenia	Rheumatoid arthritis	ACTH	100	60
DB 8 yr male	Status epilepticus with focal seizures and generalized seizures	Acquired hemolytic anemia with positive Coombs test	Cortisone	75	330
TR 14 yr male	Hypertensive encephalopathy status epilepticus	Systemic lupus erythematosus	Cortisone	300	30
LH 6 yr female	Hypertensive encephalopathy status epilepticus, residual transient paralysis	Leukemia	Cortisone	15	5
NM 13 yr female	Retention of sodium, edema, hypertension and status epilepticus	Subacute nephritis	Cortisone	300	5
SW 6 yr female	Hypertensive encephalopathy status epilepticus, coma, stupor, aphasia	Acrodermatitis chronica atrophicans	ACTH	100	5

Table 10

5 u complications of administration of adrenalectomy and cortisone in the occurrence of infectious diseases and gastrointestinal disturbances

Patient	Complication	Disease Under Treatment	Hormone (mg/100 ml)	Dose (mg/day)	Duration (days)
Infectious Diseases					
J.B. 5 y mal	Staphylococci	Streptococci	ACTH Hydrocortisone	85 114	5
M.C. 9 y mal	Cellulitis of the face	Nephrotic syndrome	Delesterone	5	
L.L. 4 y mal	Staphylococci	Nephrotic syndrome	ACTH	5	60
W.B. 8 y mal	Staphylococci	Rheumatoid	Delesterone	75	60
T.B. 24 y mal	Actinomycetosis	Nephrotic syndrome	Delesterone	100	14
S.V. 23 y mal	Staphylococci	Systemic erythematous	ACTH Cortisone	66.6 82.2	10
S.W. 6 y fem	Staphylococci	Acute arthritis	ACTH	150	5
T.H. 5 y mal	Staphylococci	Leukemia	Cortisone	60	30
J.J. 8 y mal	Streptococci	Osteomyelitis	Cortisone	100	8
Gastrointestinal Diseases					
T.B. 7 y mal	Crohn's disease	Nephrotic syndrome	Delesterone	5	10
T.G. 2 y mal	Enteritis	Nephrotic syndrome	Delesterone		10
P.T. 12 y mal	Perforated ulcer	Nephrotic syndrome	ACTH	82	0
J.C. 8 y mal	Perforated ulcer	Nephrotic syndrome	ACTH	100	10
R.A. 7 y mal	Perforated ulcer	Systemic erythematous	Delesterone	50	1
V.H. 15 y mal	Perforated ulcer	Perforated ulcer	Cortisone	56 20	100
M.M. 15 y mal	Perforated ulcer	Nephrotic syndrome	ACTH	85	1

ACTH 100 mg/day

all suffering from serious diseases. In some instances there seemed to be little doubt that the underlying disease contributed to the untoward reaction observed. However in selecting cases to discuss here I have chosen only those in which we felt that hormonal treatment was a major contributing factor and not a coincident event.

Serious consequences of treatment of sufficient severity to threaten the life of the child were encountered in 35 of the 340 children an incidence of approximately 10%.

Administration of adrenocorticotropin and cortisone may produce retention of sodium chloride and water with marked gain in weight anasarca ascites pleural effusion and pulmonary edema (table 9). Depletion of potassium may produce abdominal distention abdominal cramps weakness listlessness and lethargy. In several instances we noted it to be associated with shock. It is extremely difficult to determine the requirements of these patients for potassium. In several instances in which we have administered relatively large doses of potassium chloride the concentration of potassium in the serum has increased during the early phase of treatment to rather alarming values.

As indicated in the table convulsive seizures have occurred both associated with hypertension and in its absence. Patients with pre-existing renal disease are particularly prone to develop hypertension. Other serious complications relating to the central nervous system include status epilepticus and paranoid schizophrenia.

Probably the most frequent of the serious complications we have observed in the use of steroids has been the occurrence of severe life threatening infections (table 10). These have been particularly hazardous because they are often silent until far advanced. The most dramatic complications have involved the gastrointestinal tract. Among other complications are those involving the skeletal system. Generalized osteoporosis occasionally with pathologic fracture is particularly likely to develop in patients with rheumatoid arthritis who receive prolonged steroid therapy.

Potential dangers from use of steroid hormones

There are some serious potential dangers from the use of steroid hormones. In experimental work enhancement of the effectiveness of oncotic agents has been observed as well as successful transplantation of tumors in animals in which transplantation is otherwise unsuccessful and metastasis of tumors that otherwise will not metastasize. It is at least remotely possible that such effects of these extraordinarily potent pharmacologic agents may be manifest at a later date in humans.

In Cushing's disease there is early and often dramatic development of arteriosclerotic complications. In experimental animals

and in humans a dramatic effect on the concentration of lipids in the blood is observed. The possibility of an adverse effect on the ultimate integrity of the vascular system in patients receiving long term treatment might therefore be considered.

Typical rheumatoid arthritis of long standing and scleroderma have apparently been transformed into polyarteritis nodosa during treatment with steroid hormones.

Poststeroid syndrome

In long term therapy with large doses of delta steroid in approximately 100 m²/m in the treatment of patients with rheumatic fever serious untoward reactions developed and the dosage was gradually decreased over a period of about 80 days. These patients have regularly developed what we term "post steroid syndrome" characterized by fever, pruritus, painful nodules, fat necrosis and sometime elevated erythrocyte sedimentation rate and cardiac failure without evidence of reactivation of the rheumatic process. We thought this might be a consequence of the large dosage of hormone employed but have learned recently that other investigators have observed similar phenomena after prolonged treatment with lower doses of delta cortisone. Indeed Thomas Good and his coworkers have produced extraordinary manifestations in joints and muscles after a single large dose of delta steroid hormone.

A significant danger exists when adrenocorticotropin and cortisone or its analogs are employed in clinical pediatric practice. The danger is greatest when large doses of the hormone are used and prolonged treatment employed but is expressed in some patients as the result of treatment in relatively small dosage over a short period of time. These hormones are also potentially hazardous in ways in which clinical expression of the hazard has not been clearly established.

We are forced to recognize that in children as in adults, these drugs are extremely potent and dangerous pharmacologic agents of which we have incomplete understanding. In our opinion their use should be reserved for circumstances of real peril consisting of threat of death, threat of prolonged permanent incapacitation or threat of irreversible damage to vital tissues and organs.

Discussion

DR. DONE: Since we have not seen serious complications in patients with rheumatic fever although we have seen them 3 times

in other illnesses it was interesting that Good rarely referred to complications of therapy in patients with rheumatic fever. I wonder if he believes that the underlying disease is as important a factor as we do in deciding whether the patient is likely to develop serious complications while receiving a particular dosage.

The term poststeroid syndrome seems too broad since the phenomenon was observed only in patients who had received the delta steroids and not in patients who had received cortisone or adrenocorticotropin. I do not think there is evidence indicating that administration of other steroids is followed by similar complications.

DR H. A. GOOD: I do not know why Done's patients with rheumatic fever appeared to be somewhat 'immune' to these complications, perhaps those we see differ from those he sees. Three thousand cases of rheumatic fever were reported in Minnesota last year, but the great majority of patients, presumably with relatively mild disease, were cared for by their family physicians. In the few patients referred to us because of rheumatic fever, there has been a high mortality despite steroid therapy, perhaps then there is a difference in the form of the disease Done has been studying. However, we have certainly observed threatening consequences of steroid therapy in some of our rheumatic children: fluid retention, worsening of heart failure, frank psychosis of a paranoid schizophrenic type, convulsions and gastrointestinal ulceration have all occurred.

I agree that the poststeroid syndrome may apply only to treatment with specific steroids, perhaps the delta steroids. Although we have found that it is generally difficult to administer effective doses of the delta steroids without production of serious reactions, dosage undoubtedly is a most important factor. In experimental work with rabbits we have observed a poststeroid susceptibility to endotoxin which follows administration of either cortisone or the delta steroid.

DR C. HENRY KEMPE: From the data presented, the dosage of hormone appears to be a more significant factor in promotion of bacterial infection than does the duration of treatment.

DR R. A. GOOD: Our experience indicates that it does not take very long to develop susceptibility to infection even when administering only moderate doses.

DR VAN CREVELD: We have studied a child who has had diabetes mellitus for four years following treatment of nephrotic nephritis with moderate doses of steroids. This steroid diabetes is characterized by a light tendency to ketosis, relative insensitivity to insulin and marked decrease of glycosuria when the intake of protein is decreased.

Summary

CHAIRMAN VINCENT C. KELLEY Although much of the data presented during this conference cannot be interpreted with our present understanding of the illnesses under consideration there have been many significant contributions that will clarify our perception. Investigations of chemical and physiologic properties of beta heparin and of fibrinogen and related proteins may well point the way to a clearer understanding of the functions of connective tissue and its alterations in mesenchymal diseases. The phenomenon of micellophagocytosis and the recognition of dissociant forms of bacteria and their possible significance in pathogenesis of rheumatoid arthritis are of great interest. The demonstration of altered patterns of adrenal steroids in the plasma of patients with rheumatic fever and in unaffected siblings of these patients suggests that alteration of adrenal function occurs in rheumatic fever and may precede rather than result from the disease. Discussions of the individual mesenchymal diseases have been illuminating and the serious consequences as well as beneficial effects of therapy with steroid hormones have been vividly portrayed.

It is my hope that all of the investigators who have met here will have been stimulated by the exchange of ideas to return to their investigations with renewed vigor.

